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Radiation Bio-Effects

Summary Report
January - December 1969

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Consumer Protection and Environmental Health Service

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Radiation Bio-Effects

Summary Report
January-December 1969

William A. Mills, Ph.D., Director
Division of Biological Effects
Rockville, Maryland 20852

Compiled and Edited by
Donald M. Hodge

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Consumer Protection and Environmental Health Service
Environmental Control Administration
Bureau of Radiological Health

NOTICES

This report contains preliminary data and was prepared primarily to record the year's work, and is not intended as a substitute for publication in the open literature. It is subject to revision and does not represent a final report. Please do not quote from or refer to this work in open literature without permission from individual authors to reference the material as a personal communication.

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FOREWORD

The Bureau of Radiological Health conducts a national program to limit man's exposure to ionizing and nonionizing radiations. To this end, the Bureau (1) develops criteria and recommends standards for safe limits of radiation exposure, (2) develops methods and techniques for controlling radiation exposures, (3) plans and conducts research to determine health effects of radiation exposure, (4) provides technical assistance to agencies having radiological health programs, and (5) conducts an electronic product radiation control program to protect the public health and safety.

The Bureau publishes its findings monthly in Radiological Health Data and Reports, and in the Environmental Health Series, Public Health Service numbered publications, appropriate scientific journals, and from time to time in Division-sponsored technical reports.

The technical reports published by the Division of Biological Effects contain information generated by the Division staff which is timely and useful to the radiological health program. Subjects covered by these reports are varied, for the Division is charged with developing--through animal investigations and population studies--knowledge of the biological effects of radiation delivered to man from his environment and from his use of substances and devices that emit radiation. The reports are distributed to persons and repositories that have expressed an interest in the biological effects of radiation; in addition, the reports are available from the Clearinghouse for Federal Scientific and Technical Information.

Readers are encouraged to report omissions or errors to the Bureau. Additional comments or requests for further information are also solicited.

John C Villforth

John C. Villforth
Director
Bureau of Radiological Health

PREFACE

This annual report of the Division of Biological Effects is the first one that summarizes the investigations of the two Branches that comprise the newly formed Division; it also reflects the extension of our research effort into areas generally covered by the term nonionizing radiation. The core of the Epidemiologic Studies Branch was formerly the Population Studies Program, and the Experimental Studies Branch was previously the Rockville laboratories of the Radiation Bio-Effects Program. Together they form an integrated research institution that can investigate the effects of radiation in experimental animals and evaluate the effects of radiation in populations.

Much of the credit for establishing a well-rounded research program within the Division can be given to the excellent support of the Division's Advisory Committees, a group comprising some of the nations most outstanding experts in radiation research. The Committees played an important role in the development of continuing programs for the Division's long-term Collaborative Radiological Health Laboratory in Fort Collins, Colorado, and in initiating a long-term ^{89}Sr study using cats. We are most grateful for the thoughtful suggestions of these Committee members who are listed in Appendix C.

For the last several years a representative from the Division has attended the Bio-Medical Program Directors' meeting of the Atomic Energy Commission's Division of Biology and Medicine. This meeting with our colleagues in the AEC is one valuable mechanism by which we exchange information in many areas of radiation biology. This fall we welcomed the opportunity to host the meeting in our Rockville facility and present the Division's research activities. Activities of the Bureau's other Divisions were also summarized for this group of scientists.

The Division was active this fall in organizing the Bureau-sponsored "Symposium on the Biological Effects and Health Implications of Microwave Radiation." Our investigators have also been active in other scientific conclaves at the national and international levels. Titles of their papers prepared for presentation at symposia are listed in Appendix B. Titles of papers by Division authors that have appeared in the scientific literature during the year are listed in Appendix A.

The Division has cooperated in an advisory capacity with several other agencies during the year--the Voice of America, the Federal Aviation Agency, the National Aeronautics and Space Administration, and several components of the Department of Defense. In addition we have exchanged information and are involved in cooperative studies with other agencies or within the Department of Health, Education, and Welfare including the Food and Drug Administration, the National Cancer Institute, and the National Institute for Environmental Health Sciences. Although not reported in the Division's annual report, we have welcomed the opportunity to exchange thoughts on studies of mutual interest with our colleagues in the Bureau's three Regional Laboratories: The Southwestern Radiological Health Laboratory, Las Vegas, Nevada; the Northeastern Radiological Health Laboratory, Winchester, Massachusetts; and the Southeastern Radiological Health Laboratory, Montgomery, Alabama.

In furthering international activities in radiological health the Division cooperates with the National Academy of Science by providing medical officers to participate in the Atomic Bomb Casualty Commission program in Japan. The Commission studies the effects of radiation on the survivors of the Hiroshima and Nagasaki bombings of 1945. In other foreign countries--India and Israel--the Division supports radiobiological investigations utilizing foreign (P.L. 480) exchange.

Through its intramural studies, contracts, and contacts with researchers and other experts in the field, the Division has established a research program which seeks to understand the health implications of man's exposure to ionizing and nonionizing radiations, and to make these data available to persons responsible for developing recommendations for radiation exposure guides and standards for the public health and safety.

As Director of the Division of Biological Effects, I take this opportunity to extend my personal thanks to the staff of the Division for their patience, devotion, and overall contribution to our mission, especially since I recognize that these attitudes and efforts have been made under circumstances of considerable administrative constraints. I am truly proud of the quality and quantity of our contributions to radiological health. I particularly want to thank Mr. Donald Hodge and his staff for their effort in putting together this Annual Report.

William A. Mills

William A. Mills, Ph.D., Director
Division of Biological Effects
Bureau of Radiological Health

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DIVISION OF BIOLOGICAL EFFECTS

Dr. William A. Mills, Director

The Division of Biological Effects is the biological research arm of the Bureau of Radiological Health (Figure 1). The Division supports experimental radiobiology and epidemiology studies which contribute to public health. The investigations conducted in-house and by contract examine various aspects of short- and long-term radiation effects in several animal species and in man. These investigations produce data which contribute to the knowledge of the effects of ionizing and nonionizing radiation exposure and provide supportive health effects information for the development of radiation exposure standards.

In the ionizing area, the Division investigates primarily the effects of low doses where subtle alterations are more often the rule than the exception. The Division supports a most important study in this area by contract with Colorado State University, Fort Collins. There, the Collaborative Radiological Health Laboratory, in a proposed long-term study, investigates the possible effects of irradiation in man by examining and interpreting the biological effects of known radiation exposures delivered to the beagle at various ages including in utero. Investigators in the Rockville, Maryland, laboratory employ other animals, tissues, and cells in shorter-term investigations to study radiation effects that may be applicable to human health. Various studies of human populations having unique radiation experience suggest courses that radiation-related diseases might follow. These data are valuable in showing exposure trends that may indicate routes for improving the public health or safety.

In the area of nonionizing radiation, the Division has emphasized microwave effects. Within its limited resources, the Division supports biological investigations of microwaves using experimental animals and humans who have had unique microwave radiation exposures. A new microwave source and anechoic exposure chamber have been installed at the Rockville laboratory. This chamber will permit investigators to irradiate specimens under conditions more precisely defined than before. In addition, the Division is exploring the possibility of entering into cooperative studies with other agencies having microwave capabilities.

This year the Division actively supported the Bureau-sponsored microwave symposium held under the auspices of the Medical College of Virginia. The September meeting, "Biological Effects and Health Implications of Microwave Radiation," brought together people from the medical, biological, and engineering fields to discuss the problems of microwave radiation hazards. The meeting produced much meaningful dialogue between the various disciplines and stressed the need for well-designed experiments to answer many perplexing problems.

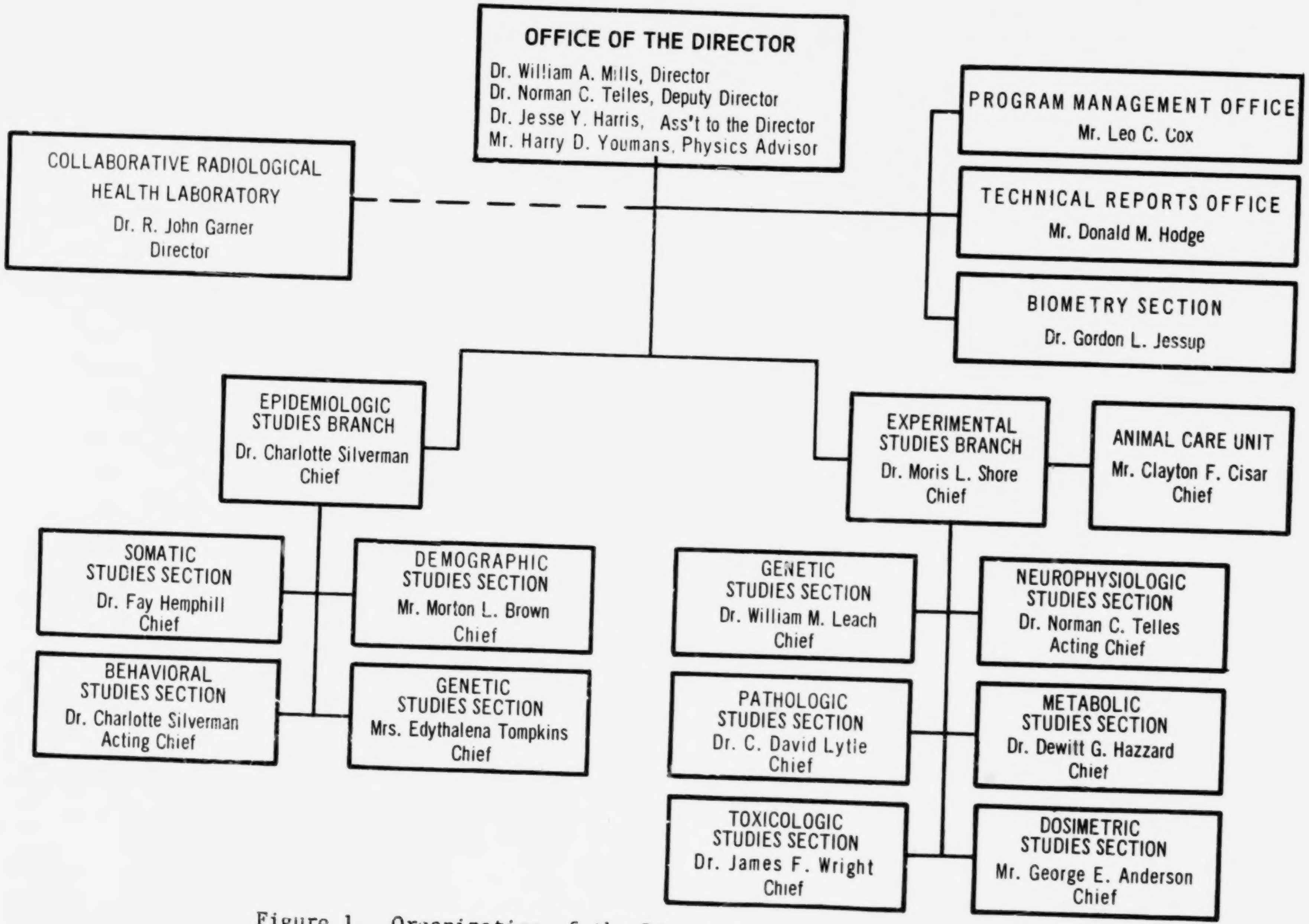
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Figure 1. Organization of the Division of Biological Effects.

BIOMETRY SECTION

Dr. Gordon L. Jessup, Jr., Chief

The Biometry Section provides statistical and mathematical consultation, experimental design, and data analysis services to all sections of the Division for a variety of radiobiological investigations. Statistical design and analysis of experimental data are essential if generalized conclusions are to be drawn and inferences made about populations from small sample results. The section also develops mathematical models to characterize physical or biological systems, and cooperates with the investigator to define experimental objectives and variables, formulate mathematical functions, define and estimate the components of variance, and synthesize number systems to characterize, compare, and evaluate individual factors and their interactions.

Results of these services are reported to the investigators in concise format as a "Biometric Analysis" which describes the statistical methods used and the conclusions that can be drawn. These reports also outline the objectives of the study and include a brief description of the experimental procedures sufficient to give the data meaning. In 1969 sixteen such analyses were prepared covering a wide range of experimental projects including radionuclide effects in cats, high and low energy X-ray effects on various species of animals and animal systems, ultraviolet effects on virus infected cells and microwave effects on cell systems. Many of the comments included in these reports were incorporated into formal publications or presentations. Biometry Section personnel co-authored three papers and are co-authors on three others currently in press.

Extensive tables have been prepared showing the sample size required to detect a true difference between two binomial populations (comparing two proportions) for various combinations of α and β probabilities. The α probability, or Type I error, is the probability of concluding that an observed difference is real when in fact there is no difference between the underlying populations. The β probability, or Type II error, is the probability of failing to detect a true difference between two populations. The power of a test to detect true differences is equal to $1-\beta$. These tables were computed by using the formula

$$n = \frac{(z_{1-\alpha} + z_{1-\beta})^2}{d^2}$$

where z is the standard normal variable and d is the difference between the arc sine transforms ($\sin^{-1}\sqrt{P}$) of the two proportions. These tables are available to investigators for use in planning experiments.

EPIDEMIOLOGIC STUDIES BRANCH

Dr. Charlotte Silverman, Chief

The entire effort of the Epidemiologic Studies Branch (formerly the Population Studies Program) is directed toward the study of human populations exposed to radiation, both ionizing and, more recently, nonionizing radiation. The research program involves the study of radiation exposure to various population groups, effects of radiation in various population groups, and factors influencing exposure and effects.

Population groups under investigation are drawn from the general population, occupational groups, persons exposed for diagnostic purposes, persons exposed for therapeutic purposes, those exposed to fallout from testing of nuclear devices, and those exposed to radiation from detonations over Hiroshima and Nagasaki.

Methods of investigation are varied. Some studies involve the analysis of morbidity or mortality statistics; others are based on data obtained by special surveys of population samples. In several studies, histories and examinations of individual members of defined population groups are obtained both for retrospective and prospective investigations. Some of the studies are conducted entirely by Branch staff, others are supported through contracts and research grants, and some combine intramural and extramural efforts.

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BEHAVIORAL STUDIES SECTION
Dr. Charlotte Silverman, Acting Chief

The Behavioral Studies Section was established during the year to emphasize and encourage further investigations into mental and behavioral effects associated with radiation exposure. Attention was drawn to this area by the preliminary findings of a grant-supported study being conducted by Dr. Roy E. Albert and associates in New York City.[1-3] They reported the surprising finding of an increase in mental illness (as well as an anticipated increase in malignancy) in a group of over 2,000 persons who had had X-ray treatments to the scalp for tinea capitis (ringworm of the scalp) when they were children. This tentative finding will be tested in a current study of children in Israel who years ago had similar radiation of the scalp for the same purpose.

Possible behavioral and other effects associated with exposure to nonionizing types of radiation led to preliminary planning for such studies. The feasibility of investigating population groups occupationally or therapeutically exposed to microwaves is being explored.

A. DELAYED EFFECTS OF SCALP X IRRADIATION
Charlotte Silverman, Project Officer

This study, initiated in September 1968, is being conducted by the Department of Clinical Epidemiology of the Tel Hashomer Government Hospital in Israel. Dr. Baruch Modan, head of the department, is the principal investigator. Mr. Sheldon G. Levin, the statistician in our program who helped to develop the study, is on leave of absence to work on the project during the developmental phase.

This is a follow-up study of immigrant children in Israel who received X-ray treatments to the scalp for tinea capitis during the period 1949-1959 when they arrived from North Africa and the Middle East. The once popular but now outmoded therapeutic procedure of X-ray epilation for ringworm of the scalp was estimated to deliver about 500 rads to the scalp and about 150 rads to the surface of the brain.

The objective of the study is to determine if late effects of such irradiation to the head can be demonstrated, particularly in the form of central nervous system changes or disorders, mental retardation, mental illness, and malignancies.

The study plan specified a one-year methodologic pretest prior to the full-scale study. During the pretest pilot year, methods were evaluated

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[1] Albert, R.E. et al. Arch. Envir. Health, 17:899-918, 1968.
[2] Albert, R.E. et al. Arch. Envir. Health, 17:919-934, 1968.
[3] Schultz, R.J. and Albert, R.E., Arch. Envir. Health, 17:935-950, 1968.

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for identifying and locating irradiated patients, selecting and locating control subjects, determining interim morbidity and mortality experience, and establishing the current health status of living subjects. In addition, dosimetric methods were evaluated for estimating radiation dose delivered to various parts and organs of the head and brain.

Original records were obtained from the treatment centers at Haifa, Tel Hashomer, Jerusalem, and Hadera, and a tinea roster established of 19,147 children. According to the records, about 10 percent had not had X-ray treatments; the total irradiated group numbered 16,473, with about equal numbers of boys and girls, most of whom were irradiated during the years 1952-1959 when they were 3 to 12 years old. More than 200 of the children received more than one course of X-ray treatment to the scalp.

For the pilot study, a random sample of 938 patients was selected, stratified primarily by number of treatments and country of origin. This was supplemented by a group of 274 nonirradiated siblings and a group of 195 non-siblings selected randomly from the Central Population Registry of Israel. Physical examinations were done on 60 subjects.

Methods developed and modified during the pretest period are applicable to the full-scale study which is scheduled to begin in 1970. The following decisions were reached about the conduct of the full-scale study:

Control Groups - Two control groups are to be used: population controls and sibling controls. The "nonirradiated tinea" cases cannot be used because there is no certainty that they had not been irradiated in their country of origin before arrival in Israel, and the "non-tinea" cases in the registry are too few in number.

Study Population - After exclusions for various reasons, there will be a group of approximately 11,000 irradiated tinea cases for study. An equal number of matched population controls and approximately 7,000 sibling controls will be selected from the Central Population Registry. The total study population will be approximately 30,000.

Study Procedures - All cases and all controls will be traced. To the extent possible, all records will be checked against hospital, tumor registry, welfare, mental hospital, police, school, and army records. A sample of about 1,000 individuals, equally divided among case and control groups, will be scheduled for physical and laboratory examinations.

Duration of Study - An extension of time of an additional year or more, beyond the estimated two years, will be needed to complete the study.

SOMATIC STUDIES SECTION

Dr. Fay M. Hemphill, Chief

The Somatic Studies Section fosters and participates in studies of human populations exposed to sources of ionizing and nonionizing radiation. Radiation-related changes in human growth and development, changes in organ function, and changes in patterns of morbidity and mortality are investigated by the Section. Data from the studies are integrated with experimental laboratory findings and related to population data.

Of several contracts in force in January 1969, one was terminated, one continued without additional funds, and one is being negotiated. One of the in-house studies active in January was terminated, one was begun in July, and the others are continuing. These studies are summarized in the sections that follow.

A. UTAH-NEVADA-ARIZONA STUDIES OF FALLOUT EFFECTS

Fay M. Hemphill, Project Officer

In 1963, testimony presented to the Joint Committee on Atomic Energy raised a number of questions concerning the possibility of thyroid injury to Utah children as a consequence of their consuming milk contaminated with iodine 131 derived from nuclear detonations at the Nevada Test Site. Subsequent studies showed no more thyroid abnormalities in children in Washington County, Utah, than among a similar group of children in Arizona.

Reexamination and follow-up of some 1,800 "exposed" and 3,000 "non-exposed" children in Utah-Nevada and Arizona examined over the period 1965-1968 showed no evidence of excess thyroid disease among the "exposed" group.

To obtain information on surgically treated thyroid disease, data were collected from all hospitals and pathology laboratories in Utah and Nevada from 1948-1967; a similar collection was continued through 1968. For the period 1948-1962 Weiss[1] stated, "Observed frequencies of thyroid cancer were compared with expected numbers derived from other incidence studies. These numbers suggest an excess in Utah most marked in the most recent five-year period. Continued study is needed to determine whether a true excess of thyroid cancer is occurring in Utah and, if so, what genetic or environmental factors may be associated with it." All available data for Utah are being studied, but have not been analyzed completely. Some information has been obtained for Nevada.

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[1] Weiss, E.S. et al. Amer. J. Pub. Health 57:1807-1814, 1967.

B. RADIATION INCIDENT REGISTRY

Fay M. Hemphill, Loren F. Mills

Consonant with objectives of the Bureau of Radiological Health for studying human effects of nonionizing radiation and with requirements of PL 90-602 for reporting the somatic and genetic effects of radiation on humans, a national registry of radiation incidents was initiated and is aimed particularly at nonionizing radiation incidents. Guidelines were developed for reporting. A contract proposal was reviewed which would supply reports of incidents from a large volume of literature not limited to the United States. The registry will compile information including the date and location of the incident, the type of facility and kind of radiation involved, and the age, sex, and occupation of the persons directly affected by the incident. The Somatic Studies Section plans to maintain the registry through FY 1970.

C. RADIATION PATHOLOGY REGISTRY

Fay M. Hemphill, Project Officer

These studies over many years developed an extensive list of case studies of tissues from many organs from patients known to have been irradiated. The body of information and teaching materials developed under the contract will remain available at AFIP (Armed Forces Institute of Pathology). Due to changes in objectives of the Bureau and restricted funds, the contract was not continued beyond October 31, 1969.

The contractor's Cumulative Progress Report as of May 31, 1969, states: "The total number of cases reviewed by the Registry of Radiation Pathology during the period 30 November 1967 to 31 May 1969 was 2,545. Each such case study includes an appraisal of the clinical aspects and indication of radiation therapy followed by a careful examination of the histological sections which may number from one to fifty or more slides. Of these cases reviewed, 733 or 29 percent were considered to have histopathologic changes characteristic of radiation effect and supported by accurate and detailed information concerning previous radiation exposure." This report gives the classification and disposition of the cases in the Registry. Some 418 were entered in the Termatrex information system from which data are quickly retrievable by sex, age, source of the case, mode of irradiation, dose, body topography of radiation therapy, anatomical site of radiation effect, clinical indication for irradiation, behavior of tumor, post-irradiation surgical procedure and post-irradiation death data.

The contractor's terminal report has not been received but more than 500 cases are expected to have been entered into the Termatrex system.

D. ELECTRONMICROSCOPY OF THE THYROID GLAND

Fay M. Hemphill, Project Officer

Studies of normal and abnormal (radiation associated) thyroid tissue have been contracted with the Washington Hospital Center, Washington, D. C. A progress report for 1969 shows that, through August, 64 sections were prepared and 15 grids were examined, 117 electron micrographs were made, and 60 of the plates were studied. A detailed report, "Ultrastructure of the Normal Human Thyroid," has been prepared for publication. This report describes the thyroid tissue from 21 persons, aged 14 to 74 years, that has been studied by light and electron microscopy.

E. MORTALITY, HEALTH INDICES, AND RADIATION CORRELATION STUDIES

Fay M. Hemphill

Data were collected from counties having records of man-made environmental radiation levels. Radiation variables included the gross beta radiation from the air sampling network; ^{89}Sr, ^{90}Sr, ^{131}I, and ^{137}Cs levels derived from the milk sampling and diet sampling networks; and the gross alpha and beta radiation from water sampling data. With annual age-specific death rates, 1960-1967, as the dependent variable, multiple regression analyses were started. Analyses completed to date are insufficient for a report, but regression coefficients of the radiation variables tested have not been statistically significant at $P = 0.05$. In other words, studies to date indicate no significant correlation between the selected radiation variables and the mortality data.

F. THOROTRAST EFFECTS ON HUMANS

Fay M. Hemphill

Thorotrast, colloidal thorium dioxide, was commonly used from 1930 to 1950 as a contrast medium for diagnostic X-ray studies. It was injected intravascularly into the patient and has been strongly implicated as the cause of liver tumors in the recipients. Because of its long half-life, high degree of retention in the body, large number of radioactive daughter products, as well as the considerable translocation of these daughter products, many experts think that, from a purely dosimetric viewpoint, Thorotrast might also increase the incidence of leukemia, bone sarcoma, and lung tumors in these patients.

The Bureau has supported previous small-scale studies that have shown the feasibility of conducting a full-scale study of morbidity and mortality patterns of persons having long-term thorium body burdens. Plans for locating a sufficient number of living or deceased persons have been proposed. Improved organ-dose studies are to be developed simultaneously with methodological studies to enable observed effects to be correlated with organ dose and years at risk.

G. DIAGNOSTIC RADIATION IN SHORT-STAY GENERAL HOSPITALS

Fay M. Hemphill, Project Officer

This study explores the trends in utilization patterns of 10 categories of radiation in selected short-stay general hospitals. Data are purchased in six-month blocks from the Commission on Professional and Hospital Activities of Ann Arbor, Michigan. The 228 participating hospitals cover more than one million hospitalizations per six-month period. Data acquired during FY 1969 for calendar years 1963 and 1966 disclosed significant shifts in category and volume of radiation utilization. Changes in each of the 10 categories of diagnostic radiation, and in combinations of the 10, provide gross indications of trends in diagnostic use of radiation. Such information is valuable in estimating exposure to the population due to medical radiation examinations in general hospitals in many areas of the United States.

From more than 2 million discharges per year from the study hospitals, the observed annual rate of increase of those receiving any diagnostic radiation approximated 1.5 percent per year. Some 1/3 and 3/5 of the increase may be attributed to "age" and "diagnosis" respectively. The remaining increase is statistically significant at $P \leq 0.01$. Similarly, there was significant increase in conjoint use of the ten diagnostic radiation categories studied. A paper, "Diagnostic Radiation Utilization in 228 Short-Term General Hospitals (United States 1963 and 1966)," has been submitted for publication.

H. ATOMIC BOMB CASUALTY COMMISSION

Charlotte Silverman, Project Officer

The Atomic Bomb Casualty Commission was established in Japan more than 20 years ago to conduct long-term studies of delayed effects on the survivors of the atomic bombs detonated over Hiroshima and Nagasaki. It is now a cooperative research agency of the U.S. National Academy of Sciences - National Research Council and the Japanese Institute of Health of the Ministry of Health and Welfare.

The U.S. Public Health Service has cooperated in this program for many years by the assignments of professional personnel. Since 1956, the Public Health Service has assigned 46 medical officers and one dental officer to the ABCC programs on two-year tours of duty; assignments have been made to the departments of pathology, medicine, and clinical laboratory (cytogenetics). During the past ten years, PHS officers have reported more than 80 studies, contributing significantly to the world literature on radiation injury and disease induction. Five PHS physicians are presently assigned to the ABCC.

In February 1969, at the annual research program review of the ABCC in Hiroshima, PHS officers reported on several studies. Thyroid disorders, the subject of extensive investigation in the autopsy series, were discussed in a new report that described the findings of 3,067 consecutive autopsies from the master sample.[1] Among the 1,096 individuals estimated to have received no radiation dose, careful sectioning of the entire thyroid gland revealed small thyroid carcinomas in 17.9 percent of the cases, compared to 1.0 to 4.0 percent in American series of similar methodology. In these cases, primary carcinomas outnumbered secondary malignancies 10:1. Radiation was associated with increased frequency of occult thyroid carcinoma discovered at autopsy in those exposed to 50 or more rads at the time of the bomb. Nodular thyroid glands were infrequently found; among 22 individuals who were less than 10 years old at the time of the bomb, only one nodular gland has been found (this is a sharp contrast to the experience of the Marshallese).

Other studies of the thyroid in the consecutive autopsy series have been presented and submitted for publication.[2,3] Occult papillary thyroid carcinomas were found to have metastasized to the cervical lymph nodes in 16 percent of 128 autopsy cases. Metastases were significantly more frequent in men than in women. The metastases were generally occult, and sometimes multiple, contralateral and bilateral. "In the entire autopsy series, only one occult thyroid carcinoma was the cause of death; 517 other persons with occult papillary carcinoma of the thyroid reached the end of their lifespan without awareness or manifestation of the presence of the tumor."[2]

A clinical study of thyroid carcinoma in atomic bomb survivors by former PHS officers and colleagues at ABCC was published this year.[4] An excess of thyroid cancer, greater in women than in men, was found among the Japanese survivors and there was a consistent, positive association between estimated radiation dose and the development of thyroid cancer. Age at time of exposure was thought to be an important factor since the association between distance from the hypocenter and increased occurrence of thyroid carcinoma, apparent in males and females under age 40 and in females over age 60, was barely perceptible among females in the intermediate years. These findings are consistent with the reported increase in thyroid cancer frequency after therapeutic radiation in early childhood.[5]

Additional papers by ABCC personnel are in various stages of preparation or submission;[6-10] papers presented orally during the year are listed in Appendix B.

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[1] Sampson, R.J., Key, C.R. et al. Prevalence of Thyroid Carcinomas at Autopsy, Hiroshima-Nagasaki. To be published in J. Am. Med. Assoc.

[2] Sampson, R.J., et al. Metastases from Occult Thyroid Carcinoma - An Autopsy Study from Hiroshima and Nagasaki, Japan. Accepted for publication in Cancer.

[3] Sampson, R.J. et al. Papillary Carcinoma of the Thyroid: Sex and Size Related Features in 525 Cases Diagnosed at Autopsy, Hiroshima-Nagasaki. Accepted for publication in Cancer.

[4] Wood, J.W. et al. Am. J. Epidemiol. 89:4-14, 1969.

[5] Hempelmann, L.H. Science 160:159-163, 1968.

[6] Robertson, J.D. et al. Cholelithiasis in Hiroshima-Nagasaki.

[7] Schreiber, W.M. et al. Primary Carcinoma of the Liver, Hiroshima-Nagasaki.

[8] Schreiber, W.M. et al. Cirrhosis of the Liver, Hiroshima-Nagasaki.

[9] Key, C.R. et al. Thyroid Carcinoma in Hiroshima and Nagasaki. 2. Thyroid Carcinoma Associated with other Primary Cancers.

[10] Gregory, P.B. Effects of Ionizing Radiation on the Skin.

I. BACKGROUND RADIATION AND CONGENITAL MALFORMATIONS

Fay M. Hemphill

A cooperative project with the University of Minnesota, to determine whether there is a relationship between natural background radiation and congenital malformations, was completed in 1967.[1] The methods used to obtain the background gamma dose rate measurements in selected cities and towns in the three study States, Colorado, Michigan, and Minnesota, were described in a publication this year.[2]

The epidemiologic and background radiation data were reexamined this year with specific attention to mongolism rates.[3] No supportable correlation between background radiation and mongolism was established. (The earlier report on all congenital malformations also failed to establish an association with background radiation).

J. DIATHERMY RELATED EFFECTS

Loren Mills, Project Officer

Persons treated with diathermy provide potential study populations for the investigation of nonionizing radiation effects. Plans are under way to determine the extent of dental diathermy practice, and the feasibility of identifying and following a study population of sufficient magnitude to evaluate any ocular effects from such treatment. Diathermy is used by dentists to induce clotting to prevent the "dry socket" sequela of tooth extractions.

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[1] Schuman, L.M. and Gullen, W.H. Background Radiation in the Etiology of Congenital Malformations (Final Report Contract SAph 76303). Division of Radiological Health, U.S. Public Health Service, September 30, 1967.

[2] Levin, S.G. and Stoms, R.K. Am. J. Public Health, 59:102-107, 1969.

[3] Schuman, L.M. and Gullen, W.H. Background Radiation and Down's Syndrome. Meeting on Down's Syndrome, New York Academy of Sciences and the National Foundation, Waldorf-Astoria Hotel, November 25, 1969.

K. FETAL IRRADIATION STUDY

Charlotte Silverman, Fay M. Hemphill, Project Officers

A long term prospective study of X-ray pelvimetry, conducted in Baltimore by the Johns Hopkins University, has been concerned with the effects of in utero radiation experience among more than 20,000 exposed pregnant women and 35,000 controls. Primary emphasis has been given to the frequency of leukemia and other malignancies in exposed and non-exposed children. Preliminary reports on the leukemia experience[1] and on cancer and other mortality[2] have been presented and a final report is to appear shortly.

Last year, attention was directed to the reproductive performances of exposed and non-exposed young women to determine whether an effect on fertility could be observed. As a result, a striking discrepancy was noted in the sex-ratio of children born to those mothers who had been irradiated prior to the third trimester of fetal life. Of 28 second generation children located through 1966, 20 were boys and only 8 girls.[3] The hypothesis was presented that non-disjunction of the X chromosome may be a mechanism producing excess male births in F_2 births to mothers exposed early in pregnancy.

F_2 pregnancies and births have now been ascertained, by exposure and year, from 1960 through 1968. F_1 and F_2 data have been put on tape, and programs for computer analysis are being prepared. Preliminary analysis indicates that more abnormalities and problems are appearing among the offspring of exposed mothers than among controls.[4]

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[1] Lilienfeld, A.M. Yale J. Biol. Med. 39:143-164, 1966.

[2] Diamond, E.L. Cancer and Other Mortality as a Sequel of Fetal Irradiation: A Prospective Study. Presented at 1st Meeting of Society for Epidemiologic Research, Shoreham Hotel, Washington, D.C., May 10, 1968.

[3] Meyer, M.B. et al. Johns Hopkins Med. J. 123:123-127, 1968.

[4] Meyer, M.B. et al. Am. J. Epidemiol. 89:619-635, 1969.

GENETIC STUDIES SECTION

Mrs. Edythalena Tompkins, Chief

The Genetic Studies Section examines the genetic effects of radiation in human populations exposed to ionizing and nonionizing radiation. For the most part these studies include long-term investigations of successive generations of people after radiation exposure. Such populations are identified and their family members, parents, and progeny examined for abnormalities that may be radiation-related. Laboratory findings, notably chromosomal studies, are integrated with population data.

A. EVALUATION OF A POSSIBLE ASSOCIATION BETWEEN INCREASED INFANT MORTALITY AND FALLOUT

Edythalena Tompkins, Morton Brown

During the year several articles were published in various journals suggesting the possibility that the decrease in rate of decline of infant mortality rates in the United States was associated with the deposition of fallout, and in particular ^{90}Sr, from the testing of nuclear weapons. All of the data which had been presented as demonstrating a correlation between fallout and infant mortality was reviewed and did not support the association.

Further evaluation of the possibility of such an association appeared necessary. The hypothesis was formulated that if fallout was affecting infant mortality, then the time of maximum change of trend of rates within states should be the same for white and non-white and for neonatal and post-neonatal mortality rates.

The assumption was made that two straight lines could adequately describe the trends of infant mortality rates for the years 1935-1967. A computer program was developed to determine the two least squares fitted lines which had the maximum difference in slope. The infant mortality data for the appropriate years for each continental state were then analyzed to determine the year of maximum difference in slope of the trends of infant mortality for white infants, non-white infants in states with more than 10 percent non-white live births, and neonatal and post-neonatal mortality rates. The results of these analyses are shown in Table 1.

The lack of consistency in time of the change in slope between the different rates within a state does not support a hypothesis of a single factor affecting the infant mortality rates.

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TABLE 1. YEAR OF MAXIMUM CHANGE OF INFANT MORTALITY RATES

State	Neonatal Mortality Rates	Post-Neonatal Mortality Rates	Infant Mortality Rates White	Infant Mortality Rates Nonwhite
Alabama	1947	1949	1952	1948
Arizona	a	a	1947	a
Arkansas	1948	1948	1951	1948
California	a	1950	1950	1950
Colorado	a	1951	1950	
Connecticut	1950	1950	1951	
Delaware	a	1951	1951	1950
District of Columbia	1949	1950	a	1950
Florida	1951	1950	1951	1950
Georgia	1949	1949	1950	1949
Idaho	1953	1951	1951	
Illinois	1951	1951	1951	1950
Indiana	1950	1953	1951	
Iowa	1952	1950	1952	
Kansas	1949	1950	1951	
Kentucky	a	1955	a	
Louisiana	1950	1950	1950	1950
Maine	1952	1952	1952	
Maryland	1951	1950	1951	1951
Massachusetts	1951	1950	1950	
Michigan	1949	1953	1949	1951
Minnesota	1951	1951	1951	
Mississippi	1950	1948	1951	1948
Missouri	1950	1950	1950	1950
Montana	1953	1950	1950	
Nebraska	a	1950	1950	
Nevada	a	a	a	a
New Hampshire	1951	1951	1951	
New Jersey	1951	1950	1950	1950
New Mexico	a	a	a	a
New York	1950	1951	1951	1949
North Carolina	1950	1948	1952	1948
North Dakota	1945	1949	1950	
Ohio	1951	1951	1950	1951
Oklahoma	a	1950	1951	1946
Oregon	a	1950	1950	
Pennsylvania	1950	1952	1951	1951
Rhode Island	1949	1950	1950	
South Carolina	1953	1949	1952	1949
South Dakota	1950	1950	1950	
Tennessee	1952	1952	1952	1951
Texas	1952	1953	1953	1950
Utah	1950	1949	1950	
Vermont	1950	1951	1951	
Virginia	1950	1950	1951	1948
Washington	a	1950	a	
West Virginia	1954	1953	1953	
Wisconsin	1951	1950	1950	
Wyoming	a	1951	a	

a. These states either had no change or more than one time of change.

B. COOPERATIVE THYROTOXICOSIS THERAPY FOLLOW-UP STUDY

Edythalena Tompkins

The Cooperative Thyrotoxicosis Therapy Follow-Up Study was initiated in 1961 to investigate the incidence of leukemia in patients who had been treated with ^{131}I for hyperthyroidism. Although several studies had demonstrated an association of acute exposure to external radiation with an increased incidence of leukemia, no data on the incidence of leukemia following exposure to low dose - low dose rate radiation were available. The increasing use of radionuclides in medicine, and particularly, the use of ^{131}I to treat hyperthyroidism (it has been estimated that over 200,000 patients have been treated with this modality), made mandatory the evaluation of the possible risk associated with this treatment. The study design was based on the ability to detect a difference in leukemia rates in patients treated with ^{131}I as compared to patients treated surgically. Data were collected in such a way that it would also be possible to study other factors which might be related to radiation such as the development of thyroid neoplasms, lymphomas and other malignancies, as well as hypothyroidism.

Investigators at 25 medical institutions in the United States and at one in England provided data for the study. At each center all patients who had received any treatment for hyperthyroidism at the institution between January 1, 1946, and December 31, 1964, were entered into the study population and a medical abstract of each patient's hospital record prepared. The details of the course of hyperthyroidism and its treatment, as well as any exposure to radiation, particularly occupational and therapeutic, and a general disease history were abstracted.

Every effort was then made to locate each patient, to obtain interim medical history, and to determine the current health status. Whenever possible, the patient returned to the referral institution for follow-up physical and laboratory evaluation, including blood smears. If unable to return, he was either examined by his local physician, interviewed by study personnel, or asked to complete a personal questionnaire. The patients were subsequently recontacted every two years and the medical history brought up to date. When it was learned that a patient had died, a copy of the death certificate was obtained. The data for all patients were collated at the Bureau of Radiological Health for data processing and analyses. When the data collection phase of the study was completed in July 1968, 36,064 patients were included in the study population. The current health status had been determined for all but 437 patients. This represents a 98.8 percent follow-up, for a mean period of about nine years.

During 1969, the processing of all of the data collected in the study was completed and analysis started.

Leukemia Incidence

In the total study population, 47 patients developed leukemia subsequent to treatment for hyperthyroidism; 18 following ^{131}I treatment, 17 following thyroidectomy, 2 following treatment with goitrogens, and 10 in patients who had been treated with both thyroidectomy and ^{131}I. Including all of the evaluated patients and all of the years of follow-up available, the final age-adjusted leukemia rates per 100,000 person years are 11 in the patients treated with ^{131}I, and 14 in patients who had thyroidectomy as treatment for hyperthyroidism. The lower observed rate of leukemia in the radiation-treated group does not support a hypothesis of an increased incidence of leukemia in hyperthyroid patients due to treatment with ^{131}I.

Incidence of Malignant Neoplasms of the Lymphatic and Hematopoietic Tissues (Excluding Leukemia)

Thirty-seven malignancies of the lymphatic and hematopoietic tissues developed post-treatment; 16 appeared in ^{131}I-treated patients and 17 in patients who had thyroidectomies. The final age-adjusted rates were 10 per 100,000 person years following radiation and 14 per 100,000 person years following thyroidectomy. Similar to the findings on leukemia incidence, the lower rate observed in the ^{131}I patients does not support a hypothesis of radiation carcinogenesis for this group of neoplasms.

Development of Thyroid Neoplasms

A total of 85 malignant thyroid neoplasms was observed in this population concurrent with or subsequent to the time of initial treatment for hyperthyroidism: 26 (1.2 per 1,000 patients) were observed in the ^{131}I population and 53 (4.6 per 1,000 patients) in the thyroidectomy group. The result of a simple comparison between the rates observed in these two groups of patients is not interpretable. A patient who is suspected of having a thyroid malignancy concurrently with hyperthyroidism is usually selected for thyroidectomy rather than treatment with ^{131}I. Even if no malignancy is suspected during palpation of the thyroid, at the time of surgery any suspicious lesions are removed. These two selection factors led to a relatively high prevalence rate of thyroid neoplasms identified at the time of thyroidectomy, and a very low incidence rate following treatment in the group treated surgically. In the ^{131}I population there is no opportunity to examine the gland at the time of initial treatment and, therefore, the prevalence rate of neoplasms at this time remains unknown. During the observation period following the administration of ^{131}I, approximately 8 years, there did not appear to be any appreciable increase in the risk of developing thyroid malignancy.

The study has confirmed previous observations in animals and man that successful treatment of the thyroid of the young with ^{131}I, the achievement of a euthyroid state, increases the risk of the development of benign thyroid neoplasms. The use of thyromimetic drugs post treatment as a preventive measure seems to minimize the risk of developing thyroid adenomas.

C. EARLY PREGNANCY X-RAY EXPOSURE STUDY

Edythalena Tompkins

Animal studies have demonstrated severe somatic effects on fetuses exposed to X ray early in gestation. Although radiologists try to avoid diagnostic X-ray exposure of pregnant females, some exposures do occur before the patient is aware she is pregnant. It is at the time of these "accidental" exposures that the fetus is presumed to be most sensitive to radiation damage.

Plans have been developed for a study which will identify women of child bearing age who have had abdominal exposures and the estimated dose to the uterus. Later contact will determine if they were pregnant at the time of exposure or if they became pregnant shortly after exposure and, if so, the outcome of the pregnancy. The pilot phases of this study will be initiated in calendar year 1970.

D. STUDY OF EFFECTS OF USING DIAGNOSTIC ^{131}I IN CHILDREN

Edythalena Tompkins

Various studies have associated exposure of the thyroid of children to relatively low levels of X ray with subsequent development of thyroid neoplasms, malignant and benign. No information is available on the possible tumorigenic properties in children of similar dose levels of ^{131}I with its lower dose rate.

Over 3,000 children who received ^{131}I for diagnostic studies before 1960 have been identified by name. The location of another 4,000 children, similarly exposed, is known. Plans have been completed for initiating a pilot study of these children in calendar year 1970 to determine the incidence of thyroid neoplasms.

E. REGISTRY OF PHYSICIANS

Edythalena Tompkins

A five-year pilot study of the feasibility of maintaining a registry of all physicians enrolled in the American College of Radiology and the College of American Pathologists was completed in 1968. It was determined that such a registry could be maintained and all of the records from the pilot study were turned over to the Epidemiologic Studies Section.

The Registry now has 7,213 radiologists and 5,693 pathologists enrolled, and approximately 350 new specialists are added to each group each year. The offices of the two Colleges make all of the contacts with the physicians and then turn over the completed forms to our office.

The forms are not identified as to respondent. The data collected include information about the types of practice in which the physician has participated, morbidity (and mortality, when applicable) information on parents, siblings, mates, and offspring, as well as the medical history of the respondent.

The data available will allow for analyses of morbidity and mortality patterns and, through them, the investigation of possible somatic and genetic effects resulting from long-term exposure to low-level ionizing radiation.

F. PARENTAL RADIATION EXPOSURE AND DOWN'S SYNDROME (MONGOLISM)

Charlotte Silverman, Project Officer

An earlier epidemiologic study of mongolism,[1] conducted in Baltimore by the Johns Hopkins University under a research grant, demonstrated an association between maternal exposure to ionizing radiation and mongolism in children. The mothers of mongoloid children were found to have had significantly increased exposure to both fluoroscopic and therapeutic irradiation prior to the birth of the child with Down's syndrome. In contrast, the fathers of the children had no unusual exposure to X rays. A surprising finding was a small but significant increase in exposure to radar among the fathers of the mongoloid children. Fathers of affected children gave more frequent histories of exposure to radar during military service.

A principal purpose of the present study by the Johns Hopkins University is to test the suggestive previous finding that radar exposure in fathers may be associated with the occurrence of Down's syndrome in their offspring. This is being done by (1) collecting data on parental exposure and military service as well as other pertinent factors for a new and independent series of mongols and their matched controls, and (2) updating and checking information on military service and radar exposure of the original series as well as the new series. The original series included the parents of 216 mongoloid children and 216 control children; to date, more than 140 cases and their controls have been identified for the new series. Concurrent chromosomal studies are being done.

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[1] Sigler, A.T. et al. Bull. Johns Hopkins Hosp. 117:374-399, 1965.

DEMOGRAPHIC STUDIES SECTION
Mr. Morton L. Brown, Chief

The Demographic Studies Section is responsible for the planning, execution, and analysis of studies of human populations exposed to ionizing and nonionizing radiation. These studies relate demographic and other relevant characteristics of the population to exposure parameters for specific sources. The Section also identifies populations at high risk as a guide to studies of somatic and genetic effects, and integrates experimental laboratory findings with related population data.

A. POPULATION DOSE FROM X RAYS, U.S. 1964
Morton L. Brown, Phyllis Segal

The 1964 X-Ray Exposure Study was conducted to estimate X-ray exposure and dose for the general population based upon the X-ray experience of a representative sample of the U.S. population. The first report on the Study, "Population Exposure to X-Rays, U.S. 1964" presented estimates of persons, visits, examinations, and films, according to demographic and socioeconomic characteristics of the population and by types of facilities and specialities of the practitioners. Estimates of exposure at the skin associated with selected types of examinations were included.

The final report of the 1964 X-Ray Exposure Study, "Population Dose from X-Rays, U.S. 1964" was published in October of this year. This report describes the dose models develped for the Study and presents estimates of gonad and genetically significant dose for the U.S. population in 1964. The following is a summary of selected data from the dose report.

Estimation of gonad dose due to diagnostic roentgenology involved the development of two separate models. The model for estimating the gonad dose received during radiographic examinations was developed at the Johns Hopkins University. Exposure data derived from an experimental setup were in the form of 600 X-ray scans (20 scans on each of 30 individuals selected to match the range of heights and weights of persons in the Study). The response of each of seven detectors, placed at selected intervals over the body surface, was recorded in digital format as each cell in a matrix of 3,000 2- by 2-cm cells was exposed. Each matrix covered an area 60 cm by 200 cm, corresponding to the dimensions of the tabletop. An indirect computer dosimetry system was then utilized to yield estimates of exposure to the gonads which were subsequently converted to estimates of absorbed dose.

In order to evaluate the dose model, exposures were measured directly at the Department of Radiology at the Hospital of the University of Pennsylvania. For each examination included in this test series, information identical to that collected in the X-Ray Exposure Study was obtained. This information was processed using the same procedures as those used for the X-Ray Exposure Study to produce exposure estimates for input to the dose model. A comparison of mean differences indicated good agreement between the calculated estimates and the direct dosimetry.

The model for estimating gonad dose received during fluoroscopic examinations was developed at Emory University. A phantom was used to experimentally determine the percentage contribution to gonad exposure that results from a known exposure to a given surface unit area. Tables representing these percentage contributions were generated and estimates of radiation dose to the gonads were then derived from these tables and collated with the measurements of surface exposure determined in the survey by means of the fluoroscopic record pack. The final gonad exposure estimates were then converted to estimates of absorbed dose.

A series of tests was also carried out to evaluate the fluoroscopic dose model. Exposure estimates produced by the model were compared with those determined dosimetrically during a simulated fluoroscopic examination. Mean values of the model exposure estimates, when compared with the dosimetrically determined estimates, were found to lie within the limits of error expected for the dose model.

Estimated mean gonad doses for selected types of roentgenologic examinations are shown in Table 2. Radiographic examinations of the abdomen and pelvis produced the highest gonad doses; examinations of the head, neck, and extremities produced low gonad doses.

Testicular doses resulting from radiographic examinations of the abdomen and pelvis reflected the impact of beam sizes significantly larger than the minimum size needed to expose the film. Fluoroscopic examinations produced ovarian doses which were higher than testicular doses from the same types of examinations. This finding reflects the fact that field sizes used in fluoroscopy usually exclude the testes but not the ovaries from the direct beam. It was possible, because of the design of the Study, to simulate estimates of gonad dose under the assumption that all examinations in the Study had been conducted with beam sizes no larger than the film size. This resulted in a marked reduction of testicular doses.

In general, the smallest ratios of beam area to film area were found in hospitals; this may reflect a greater concern and attention to the need for close collimation and the availability of specialized equipment. Examinations done in the private offices of physicians other than radiologists tended to show the largest ratios, possibly reflecting use of equipment which does not provide adequate collimation, and a somewhat lower level of training on the part of the technologist or other operator of the X-ray machine.

TABLE 2. ESTIMATED MEAN GONAD DOSE PER EXAMINATION BY TYPE OF EXAMINATION AND SEX, UNITED STATES, 1964

	Millirads per Examination		
Type of Examination	Male	Female	Fetus
Skull	a	4	7
Cervical spine	8	2	3
Chest			
Radiographic	5	8	6
Photofluorographic	a	8	8
Fluoroscopic	1	71	b
Thoracic spine	184	9	9
Shoulder	a	a	a
Upper gastrointestinal series			
Radiographic	130	360	343
Fluoroscopic	7	198	198
Barium enema			
Radiographic	1,535	439	417
Fluoroscopic	50	366	397
Cholecystography or cholangiogram	2	193	206
Intravenous or retrograde pyelogram	2,091	407	451
Abdomen, KUB, flat plate	254	289	291
Lumbar spine - lumbo-sacral	2,547	420	239
Pelvis or lumbo-pelvic	717	41	82
Hip	1,064	309	366
Upper extremities	2	1	b
Lower extremities	96	a	a
All other			
Radiographic	52	92	251
Fluoroscopic	229	a	a

a. Quantity less than 0.5.

b. Figure does not meet standards of reliability or precision.

With the estimated gonad doses for both radiographic and fluoroscopic examinations, it was possible to calculate the genetically significant dose. GSD may be defined as the gonad dose which, if received by every member of the population, would be expected to produce the same total genetic effect on the population as the sum of the individual doses actually received. It can be expressed algebraically as:

$$\text{GSD} = \frac{\sum D_i \hat{N}_i P_i}{\sum N_i P_i}$$

where:

D_i = the average gonad dose to persons age i who receive X-ray examinations,

N_i = the number of persons in the population of age i who receive X-ray examinations,

P_i = the expected future number of children for a person of age i, and

N_i = the number of persons in the population of age i.

The GSD for the United States for 1964 was estimated to be 55 millirads per person, per year. This estimate is higher than that in other countries but a number of factors influence the genetically significant dose, the most obvious being the examination rate in each country. When the GSD is divided by the examinations per 100 population, the United States estimate is found to be closer to values in other countries. Table 3 compares the annual genetically significant dose in selected countries.

TABLE 3. COMPARISON OF REPORTED ANNUAL GENETICALLY SIGNIFICANT DOSE FROM DIAGNOSTIC RADIOLOGY (SELECTED COUNTRIES)

	Genetically Significant Dose in Millirads	Examinations per 100 Population	Genetically Significant Dose per Examination
United States (1964)	55	53	1.04
Sweden (1955)	38	29	1.31
Japan (1960)	39	41	.95
Denmark (1956)	22	24	.92
Great Britain (1957)	14	28	.50
New Zealand (1963)	12	37	.32

The contribution to the genetically significant dose from radiography was 96 percent, with 4 percent from fluoroscopy and less than 1 percent from photofluorography. As seen in Figure 2, males 15 to 29 years of age contributed most to GSD. Figure 3 shows that a few high dose examinations, such as lumbar and lumbo-sacral spine, barium enema, intravenous or retrograde pyelogram, pelvic, and abdominal examinations are the principal contributors to GSD.

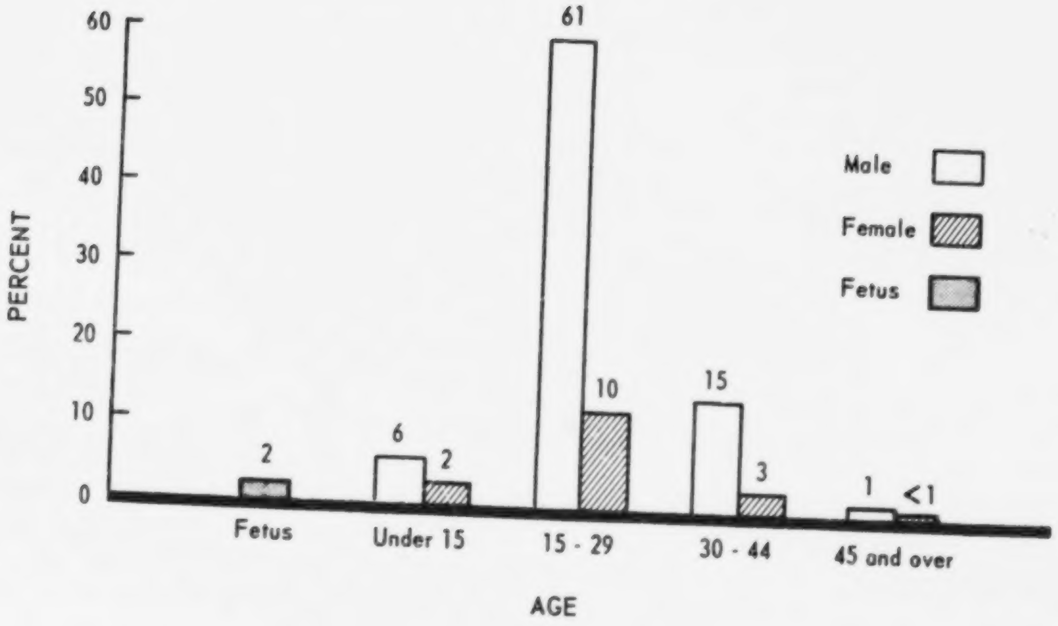

Figure 2. Estimated Percentage Distribution of Genetically Significant Dose from Diagnostic Medical Roentgenology by Age and Sex, United States, 1964.

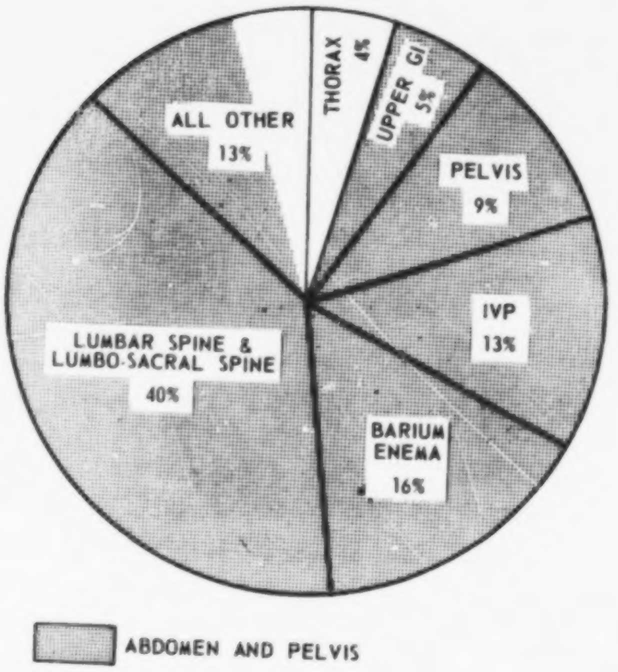

Figure 3. Estimated Percentage Distribution of Genetically Significant Dose by Type of Medical Roentgenologic Examination, United States, 1964.

When gonad doses resulting from the hypothetically reduced beam sizes mentioned previously were used in the calculation of genetically significant dose, a reduction from 55 millirads per person, per year to 19 is obtained. These reductions in genetically significant dose reflect only the impact of improved collimation and do not represent the maximum reductions possible through other improvements in radiographic technique.

B. STATISTICAL PROJECTIONS OF GENETICALLY SIGNIFICANT DOSE TO THE U.S. POPULATION

Norman S. Kessner, Morton L. Brown

A statistical model was developed for estimating the genetically significant radiation dose to the U.S. population attributable to medical X rays, for the period 1965 to 1990. The results of the model represent statistical projections, not predictions. Nevertheless, they identify areas in which program efforts may yield the most success in limiting an increase in genetically significant radiation dose, and can serve as a benchmark against which the success of program efforts can be measured.

The measure of radiation dose used in this study was the "Genetically Significant Dose" (GSD), which may be defined as the radiation dose to the reproductive organs which, if received by every member of the population, would be expected to produce the same total genetic injury to the population as the sum of the individual doses actually received. The formula for the GSD is shown in Figure 4.

$$\text{GSD} = \frac{\sum_{ij} \hat{N}_{ij}^{(\text{male})} P_i^{(\text{male})} D_{ij}^{(\text{male})} + \hat{N}_{ij}^{(\text{female})} P_i^{(\text{female})} D_{ij}^{(\text{female})} + \hat{N}_{ij}^{(\text{fetus})} P_i^{(\text{fetus})} D_{ij}^{(\text{fetus})}}{\sum N_i^{(\text{male})} P_i^{(\text{male})} + N_i^{(\text{female})} P_i^{(\text{female})} + N_i^{(\text{fetus})} P_i^{(\text{fetus})}}$$

where $\hat{N}_{ij}$ = Number of examinations of type j given to persons in the population of age i

P_i = Expected future number of children for a person of age i

D_{ij} = Average gonad dose to persons age i who receive X-ray examination j

N_i = Number of persons in the population of age i

Figure 4. Calculation of Genetically Significant Dose.

The basic model used to project genetically significant dose is shown in Figure 5 . This model applies an annual rate of increase for each type of examination to the 1964 examination frequency to obtain projected examination rates for various future years. The projected future doses for each examination were obtained by assuming linear reduction from the 1964 dose levels to a set of theoretical minimum doses. Using this model, the projected genetically significant dose was calculated for 1965, 1970, 1975 and 1990. A separate series of projections was calculated for each year, based on a high, medium and low set of rates of annual increase in examination. Projections were first made on the assumption of no reduction in dose whatsoever. Values of the genetically significant dose were also calculated assuming linear reduction in dose levels within a period of time varying from one to 26 years.

$${}^{cy}\text{GSD} = \frac{\displaystyle\sum_i\sum_j {}^{cy}N_i^{\text{Male}}(1+R_i^j)^{cy-1964}\left[{}^{1964}D_i^j - \frac{(cy-1964)}{n}\left({}^{1964}D_i^j - {}^{\min}D_i^j\right)\right]^{*} A_i^j\, P_i^{\text{Male}} + {}^{cy}N_i^{\text{Female}}(1+R_i^j)^{cy-1964}\left[{}^{1964}D_i^j - \frac{(cy-1964)}{n}\left({}^{1964}D_i^j - {}^{\min}D_i^j\right)\right]^{*}\left[A_i^j\, P_i^{\text{Female}} + (AP)_i^j C_i P_{\text{Fetus}}\right]}{\displaystyle\sum_i N_i^{\text{Male}} P_i^{\text{Male}} + N_i^{\text{Female}} P_i^{\text{Female}} + N_i^{\text{Female}} C_i P_{\text{Fetus}}}$$

* But not less than ${}^{\min}D_i^j$

Where:

${}^{cy}\text{GSD}$ = The GSD in calendar year cy

${}^{cy}N_i$ = Number of persons in population of age i in calendar year cy

R_i^j = Annual rate of increase in examination rate for exam j and age i

A_i^j = 1964 rate of examination j in age i

${}^{cy}P_i$ = Expected future number of children for a person of age i in calendar year cy

${}^{cy}D_i^j$ = Average gonad dose to persons age i who receive X-ray examination j in calendar year cy

n = Number of years to achieve minimum dose

${}^{\min}D_i^j$ = Minimum theoretical dose for exam j in age i

$(AP)_i^j$ = Ratio of exams for pregnant compared to nonpregnant women for exam j in age i

${}^{cy}C_i$ = Proportion of women in age i who are pregnant

* But not less than ${}^{\min}D_i^j$

Figure 5. Model for Projection of Genetically Significant Dose.

The 1964 frequencies of examinations and the average gonad dose for each examination were derived from the Public Health Service 1964 X-Ray Exposure Study. The set of theoretical minimum doses was derived from these same data, and was based on the assumption that all X-ray beam dimensions were reduced to a size not greater than that of the film being used for each exposure. Other needed demographic data were obtained from published sources issued by the U.S. Bureau of the Census and by the National Center for Health Statistics, PHS.

Rates of increase in examinations used in the model were obtained from a comparison of examination rates in 1964 with those estimated in an earlier survey for 1961. These rates of increase were adjusted for examinations of the abdominal area based on more recent data obtained from selected large hospitals. The schedule of rates of increase so obtained was further adjusted to yield an overall increase of 6 percent, 4-1/2 percent, and 2-1/2 percent per annum for the high, medium, and low series respectively. The 6 percent rate of increase is consistent with national data on X-ray film production suitably adjusted for imports and exports. The 4-1/2 percent rate of increase is that derived from the direct comparison of the 1964 and 1961 Public Health Service studies. The lowest level was chosen as a theoretical minimum which would be consistent with a substantial but unlikely slowing in the per capita rate of X-ray usage. A maximum limit was placed on all projected examination rates such that these could not exceed the corresponding 1964 rate by more than a factor of 10. Projected values of GSD are shown in Table 4 for various years of projection, number of years required to achieve minimum gonad doses, and level of rate of increase in examinations. If minimum doses are achieved within a 26-year period, the GSD for 1990 is projected to be 49, 77 or 105, for the low, medium and high rates of increase in examination rates, respectively. If the current schedule of gonad doses is continued, these values become 174, 291, and 412 respectively, representing in each case approximately 4 times the estimated minimum GSD.

The effect of population distribution and expected future children on the projected GSD is only 15 percent of the increase shown in Table 4 and, it is therefore unlikely that even a marked change in population dynamics will result in a significant impact on GSD. Of major importance, however, is the effect of rate of increase of examination frequencies on the GSD. Table 5 indicates that for 1990, the estimated GSD more than doubles between the low rate of increase assumption and the high rate of increase. The effect is less marked for earlier years.

All of the foregoing projections were based upon a limit on the growth of examination rates; no examination frequency for 1990 was permitted to exceed an arbitrary limit of 10 times the corresponding number for 1964. The effect of this limit is shown in Table 6, where it may be observed that it results in a GSD for 1990 approximately 5 percent less than that which would be obtained without such a limit. For comparison purposes the results of a similar limit of 5 times the 1964 rate are also shown in Table 6.

TABLE 4. VARIATION IN GENETICALLY SIGNIFICANT DOSE BY NUMBER OF YEARS REQUIRED TO ACHIEVE MINIMUM GONAD DOSE, ACCORDING TO RATE OF INCREASE IN EXAMINATIONS (GSD in millirads).

Calendar Year of GSD	Number of Years Needed						
	1	6	11	16	21	26	Never
			(High Rate of Exam Increase)				
1965	20	56	59	60	61	61	63
1970	29	29	59	73	79	83	100
1975	44	44	44	76	97	109	161
1990	105	105	105	105	105	105	412
			(Medium Rate of Exam Increase)				
1965	20	56	59	60	61	61	63
1970	28	28	57	68	75	78	94
1975	38	38	38	66	83	93	140
1990	77	77	77	77	77	77	291
			(Low Rate of Exam Increase)				
1965	19	54	57	59	59	59	61
1970	24	24	49	59	64	67	81
1975	31	31	31	51	65	72	105
1990	49	49	49	49	49	49	174

TABLE 5. EFFECT OF OVERALL RATE OF INCREASE IN EXAMINATION RATES ON GSD (Excl. Effect of Population and Future Children - GSD in millirads).

Year	GSD for Year Assuming Overall Rate Of Increase is		
	High (6.0% per Annum)	Medium (4.5% per Annum)	Low (2.5% per Annum)
1965	63	63	61
1970	95	89	77
1975	139	120	88
1990	365	256	150

TABLE 6. EFFECT OF LIMIT ON INCREASE IN EXAMINATION RATES (Assuming No Dose Improvement - GSD in millirads).

Year	GSD if 1990 Exam Rate Limited To 5 Times 1964 Exam Rate	GSD if 1990 Exam Rate Limited To 10 Times 1964 Exam Rate	GSD if No Limit is Used
		(High Rate of Exam Increase)	
1975	124	161	171
1990	293	412	439
		(Medium Rate of Exam Increase)	
1975	122	140	140
1990	278	291	294
		(Low Rate of Exam Increase)	
1975	104	105	105
1990	172	174	174

Since the trends in each of the major variables were independently estimated, no allowance was made for the impact of changes in one variable upon those in another. Evidence of this independence of change may be seen in Table 4, where in certain time periods the GSD appears to decrease before increasing again. This decrease results from a projected decrease in average doses at a rate somewhat more rapid than the increase in examination frequencies.

No assumption was made regarding the possible impact of radical changes in health care patterns within the U.S. If such changes occur, they would significantly affect the projections. In regard to minimum gonad doses, it should be borne in mind that these minimums are based solely on restriction of beam sizes to the size of receptor film. No allowance has been made for additional improvements which may reasonably come about through improved equipment design and improved radiologic techniques.

From the data developed by this project, it appears that the observed growth in examination rates and the continued high levels of gonad doses may soon produce a GSD level which is substantially above any value now known for any country, and well above that attributable to natural background. Presently, the GSD from medical X ray is estimated to be about one-half that due to natural background; by 1990 the GSD could be from 2 to 4 times that due to natural background.

C. COMPARISON OF UNITED STATES AND FOREIGN GENETICALLY SIGNIFICANT DOSE FROM DIAGNOSTIC X RAYS

Paul J. Brennan

This study was conducted to compare estimates of the genetically significant dose (GSD) calculated for the U.S. population[1] with those calculated using statistical components from Sweden,[2] Denmark,[3] and New Zealand.[4] The U.S. estimates were prepared by interchanging in the GSD calculations the four components, namely population distribution, birth rate, X-ray examination frequency, and mean gonadal dose from the four countries.

Such calculations show the effect the four parameters could have on the GSD in this country. The trend of the GSD could be estimated by comparing future corresponding U.S. components with those used in these calculations. As indicated in Table 7, the Swedish birth rates and frequency of examinations cause a much greater difference in GSD than do population distributions which account for only about a 15 percent change. The Danish population distribution and birth rate components produce a smaller difference in GSD than do examination frequency or mean gonadal dose. Although both of these components are important in determining GSD differences, the latter produces the greatest difference. The mean gonadal dose is more responsible than the other components in producing GSD differences noted in the Table for New Zealand. This component causes about 95 percent of the difference between the U.S. GSD of 55 millirads per person per day and the New Zealand value of 12.

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[1] Population Dose from X-Rays, U.S. 1964 (PHS 2001). U.S. Government Printing Office, Washington, D.C. 20402, $1.25, 144 p. October 1969.

[2] Larsson, L.E. Acta Radiol. Suppl. 157, 1958.

[3] Hammer-Jacobsen, E. Acta Radiol. (Diag.) Suppl. 222, 1963, p. 1-273.

[4] Williamson, B.D.P., [illegible] McEwan, A.C. The Genetically Significant Radiation Dose to the Population of New Zealand from Diagnostic Radiology. Christchurch, New Zealand, Dept. of Health, 1964.

TABLE 7. THE EFFECT ON GENETICALLY SIGNIFICANT DOSE ESTIMATES[a] FOR THE U.S. PRODUCED BY SUBSTITUTING INTO GSD CALCULATIONS COMPONENTS FROM SELECTED FOREIGN COUNTRIES

		Estimates Produced By Substitution			
Country	Calculated GSD[b]	Population Distribution	Birth Rate	Examination Frequency[f]	Mean Gonadal Dose[g]
U.S.	55	55	55	55	55
Sweden	38	66[c]	105	39	73
Denmark	22	63[d]	53	34	18
New Zealand	12	58[e]	50	56	17

a. Millirads per person per day.
b. See Section B, p. 28.
c. 1956 United Nations Demographic Yearbook.
d. Report data plus 1957 U.N. Demographic Yearbook.
e. Report data plus 1953 U.N. Demographic Yearbook.
f. Numbers of examinations were obtained directly from report, then combined to give frequencies.
g. Obtained from weighted average combination of corresponding examination.

EXPERIMENTAL STUDIES BRANCH

Dr. Moris L. Shore, Chief

The Experimental Studies Branch plans, conducts, and supports an experimental and multidisciplinary research program including supportive dosimetry studies, to determine the biological effects of ionizing and nonionizing radiation. It provides experimental radiobiology support to all other Bureau Divisions and maintains liaison with pertinent national and international organizations.

The Office of the Chief provides scientific direction to, and coordinates the activities of the six Sections which comprise the Experimental Studies Branch. It additionally provides, through the Animal Care Unit, services to the entire staff of ESB.

This past year has seen significant effort devoted to the examination of biological hazards of electronic product radiation as required by the Radiation Control for Health and Safety Act of 1968, P.L. 90-602. A significant level of effort has been maintained in the investigation of the biological effects of 250 kVcp X rays. A research program has been developed to examine the relative biological hazard of low energy X rays. Reprogramming has permitted the initiation of a productive research effort to investigate the biological consequences of exposure to microwave radiation. The latter two areas of investigation are timely because of the increasing concern related to radiation produced by color television and microwave ovens--electronic products which have found or will find extensive use among the general population.

Continuing attention is being devoted to the age sensitivity problem including studies of in utero radiation, to define critical periods of development when radiation may be particularly effective in producing deleterious effects. Our cat colony is an important factor in this research which has elicited significant interest from the National Cancer Institute.

Within the framework of our program, we are developing areas of investigation that examine the biological hazard of multiple environmental insuits involving radiation chemical, viral, bacterial, fungal and other agents. This approach recognizes that in his environment, man is rarely if ever, exposed to a single biological hazard. We live in a sea of electromagnetic radiation, are exposed simultaneously to multiple viral, bacterial, fungal and other agents in the environment, and subjected to the possible hazard of a multitude of pesticide residues, chemical additives, and supplements in our food. While any one environmental insult may not be associated with an unacceptable risk to public health and safety, the possible synergism of multiple environmental insults has not been adequately investigated.

The development of our research effort on the neurophysiological effects of microwave radiation has been hampered by the difficulty in identifying a suitable scientist for this program. Vigorous efforts are being made to locate and attract such an individual to our Laboratory. This area of investigation is crucial, in view of our commitments under P.L. 90-602 and the current controversy related to the subtle effects of microwave radiation reported by Soviet scientists. It is of particular concern to us that recommended levels of exposure for microwaves in this country are 1,000 times higher than those permitted in Russia. It would be imperative, for the protection of public health and safety, that we examine critically the scientific basis for the Russian microwave standard. Neurophysiological and behavioral studies are an element essential to the critical examination of Soviet work in this area. Neurophysiological and behavioral studies will require not only the recruitment of competent personnel, but also the purchase or use of relatively expensive equipment of a type not currently available to us.

There has been increasing recognition of our Laboratory as a significant center of research on the biological effects of electromagnetic radiation. Thus we have been involved in collaborative studies with the National Institutes of Health, and have been active consultants to the United States Information Agency in their contracted program of research on the biological effects of exposure to radiofrequency radiation. We are currently investigating further possibilities for collaboration with the Department of Defense in the areas of microwave bioeffects research, and research on the biological effects of exposure to magnetic fields.

We have obtained valuable assistance in developing a microwave radiation facility within our Laboratory from NASA, who made available to us a large supply of anechoic material essential for the microwave exposure facility.

Extension of the research effort of the Experimental Studies Branch is also being effected by the use of the P.L. 480 Foreign Currency Award Program. Thus, we are presently embarking on a 3-year program of research on the effects of X irradiation, including low energy X rays, on the nervous system. This program will be performed by Dr. Asim Jamakosmanovic and his staff at the University of Sarajevo, Yugoslavia.

Additional use of the P.L. 480 mechanism is being explored to provide detailed reviews of Eastern Block scientific research on the biological effects of electromagnetic radiation. Hopefully, such detailed and extensive reviews--by scientists most immediately familiar with this research--will be of immeasurable value to scientists and Public Health authorities in this country.

The staff of the Experimental Studies Branch has participated significantly in a number of scientific meetings and symposia including the Hanford Symposium on "Radiation Biology of the Fetal and Juvenile Mammal", Richland, Washington. The specific details on other participation by members of our scientific staff will be given in the reports of the various Sections of this Branch.

Members of this Branch have prepared a number of staff papers on the biological effects of electromagnetic radiation and sound which provide relatively brief orientations to the literature which has been published in these areas. Additionally, our staff has responded significantly to its responsibility, under P.L. 90-602, in the development of scientific bases for performance standards for electronic products.

While current levels of budgetary support do not permit it, expanded levels of research effort will be required to implement the provisions of P.L. 90-602. Intramural competence and productive research will be a necessary ingredient in such expanded effort. However, it is clear that extramural research will have to play a significant role in developing information necessary to support reasonable performance standards to protect public health and safety. Our staff has been active in the identification of competence and interest, within the scientific community, for research in this area. We recognize the need for that balanced program of intramural and extramural research that will permit effective implementation of the provisions of P.L. 90-602.

ANIMAL CARE UNIT

Mr. Clayton F. Cisar, Chief

The Animal Care Unit provides animals and animal care for the studies conducted by the Division of Biological Effects at the Rockville, Maryland, laboratory. The Unit assists the investigators in planning the space, the most suitable animal species or strain, and the special breeding or handling required for a particular study.

One part of the Animal Care Unit provides animals for relatively short-term studies; the other part, operated by contract, cares for animals in long-term experiments. During the year, the Unit bred and raised about 3,000 Chinese hamsters, and cared for approximately 4,000 rats, 500 mice, 60 swine, 50 rabbits, 30 guinea pigs, 15 dogs, and a few cats and monkeys.

The general health of the animals has been excellent, owing to the strict hygienic discipline maintained in the colony by the animal caretakers. Good animal health is mandatory because the subtle effects occurring in irradiated animals may be overshadowed by ill health.

The Animal Care Unit over the past several years has been involved in developing a successful and practical method for breeding Chinese hamsters. These animals have desirable cytogenetic characteristics and a notable lack of diseases and parasites.

Chinese hamsters breed satisfactorily when three males and three to five females are placed together in a breeding cage at weaning. The research colony, with an average of 550 females and 450 males -- half of breeding age, produces approximately 400 offspring from 60 litters per month. The Unit's production records show that Chinese hamsters can be produced without elaborate equipment or involved breeding and rearing methods.

TOXICOLOGIC STUDIES SECTION

Dr. James F. Wright, Chief

The Toxicologic Studies Section (formerly the Radionuclide Toxicology Laboratory) including the Outdoor Cat Colony, was moved during 1969 from Cincinnati, Ohio, to Fairfax County, Virginia. New facilities were constructed for the colony prior to the move. The new location also makes possible the services of scientists at the Division's Rockville, Maryland, Laboratory.

The major research project for the section remains as before, the late biological effects of ^{89}Sr in the cat, with special emphasis given to radiation associated hematopoietic and bone abnormalities. The research objectives are the following:

1) To identify age at which the animal is most sensitive to the effects of ^{89}Sr, from exposures beginning at conception, birth, weaning, puberty, and adulthood.

2) To determine the radiation dose delivered to the bone, bone marrow, and other organs in animals exposed at the various ages.

3) To assess the role of viral agents in cases of neoplasia of the hematopoietic or skeletal systems and their possible relationship to whole-body burdens of radiostrontium.

4) To establish if a relationship exists between radiation doses determined to be oncogenic in dogs and swine exposed to ^{90}Sr and those producing similar abnormalities in cats exposed to ^{89}Sr.

Investigations of the biological consequences of exposure to several radioisotopes of strontium have been carried out on many species of lower animals from single dose exposures to continuous lifetime ingestion of the radioisotope. In most of these studies, the incidence of osteosarcomas was increased and many also reported an increased incidence of hematopoietic neoplasms. In one large experiment, beagles on long-term exposure to ^{90}Sr from gestation to puberty have shown dose-related increase of myeloproliferative disease, granulocytic leukemia, and bone tumors. The natural incidence of all these pathologic conditions is very low in this breed. The domestic cat, however, has not been used as an experimental animal for evaluating the long-term effects of exposure to bone-seeking radionuclides. The cat is somewhat unique among domestic animals for several reasons that have a direct bearing on an experiment involving bone marrow irradiation; i.e., bone marrow disorders in the cat are more common than previously thought, myeloproliferative disease (reticuloendotheliosis) and abnormal erythrogenesis are naturally occurring diseases in the cat, and a greater variety of leukemias have been reported in the cat than in other domestic animals.

Several groups of investigators have recently confirmed a feline virus-induced lymphocytic leukemia while others have shown similar-type viral particles associated with feline myeloproliferative disease. Since myeloproliferative disease has been produced by radiostrontium in other species, the presence of these naturally occurring conditions in the feline offers a unique situation with respect to the interaction of irradiation and viral agents.

A. FELINE COLONY

Ezra Berman

There were three main objectives for this past year:

1) To relocate with minimal change in character of the colony

2) To maintain top colony production in preparation for the ^{89}Sr effects study

3) To investigate parameters which may prove important to the ^{89}Sr effects study.

Relocation of the Feline Colony

The colony, formerly in Cincinnati for six years, was relocated at Hazleton Laboratories, Inc., at Falls Church, Virginia. From November 1968 through March 1969, a feline facility was constructed at the Falls Church site. The facility (see Figure 6) is similar to the one which had existed at Cincinnati. The basic changes included centralization into a single compact perimeter, a distinct separation from other existing buildings, a support building specifically designed to the colony and experimental needs, and an impervious, cleanable floor surface throughout the facility.

The run area is divided into three functional sections: A 20-pen exposure run (in which the cats are housed during and just after radioisotope administration); a 40-pen production run (in which queening, nursing, weaning, and post-weaning cats are maintained); a 40-pen holding run (in which, at present, mature cats are maintained, but is meant to and will contain post-exposure animals). Each run is separated from the others by at least a 9-foot gravel or concrete division and a series of fences and gates. Each run contains its own houses and daily maintenance equipment and supplies. Each run is supplied with cold water, electrical power receptacles in the central work alleys, and zoned variable electric power

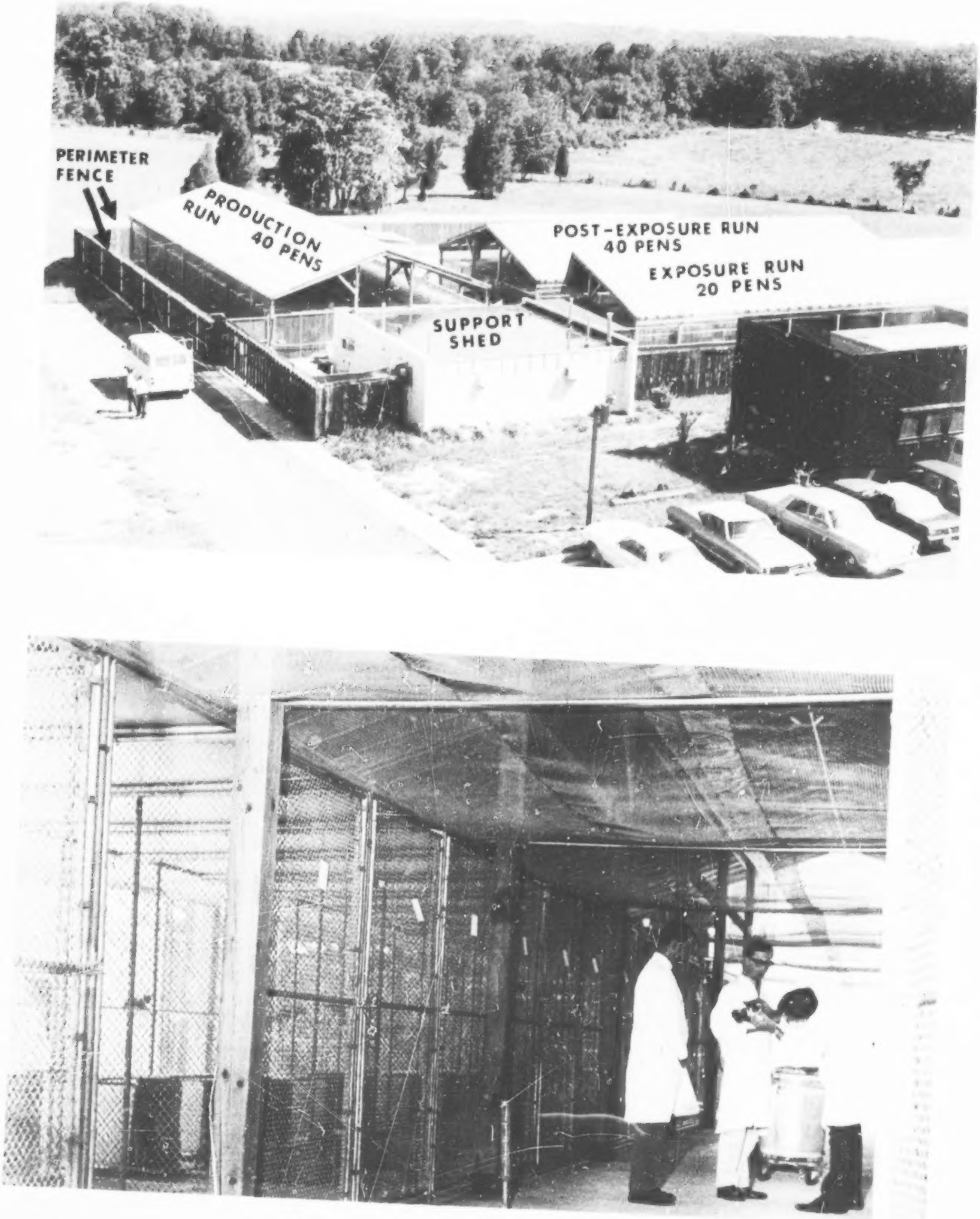
PERIMETER FENCE
PRODUCTION RUN 40 PENS
POST-EXPOSURE RUN 40 PENS
EXPOSURE RUN 20 PENS
SUPPORT SHED

Figure 6. The Toxicologic Studies Section's Outdoor Feline Colony. (Top) Production and exposure runs; (bottom) central alleyway in production run.

receptacles for heating-pad control. Each line of 20 pens in a run is backed by a gutter which leads into a system of drainage pipes, controllable in flow rate and direction, leading to four 1,500 gallon storage tanks, and also, directly to the main storm sewer. Each pen, 4 x 12 x 7 feet constructed for maintenance of up to 4 adult cats, contains a feline house (2 x 2 x 3 feet), a litter box, feed and water pans, and a 1 x 4 foot resting board 5 feet above the ground surface. The base is 4-inch-thick concrete, supporting the pen enclosures and the roof supports. The roof is peaked 20 feet above the base, sloping to 8 feet at the eaves; the peak runs the entire length over the mid-line of the work alley.

The facility, as constructed, affords free passage of air (to the purpose of dilution of infectious organisms), convenient sanitation, control of radioactive effluent, (see p. 51) separation of experimental areas, retention of animals at the facility, and prevention of entrance of stray cats into the facility. The support shed is supplied with heated water, electrical power, and air conditioning. The shed is segregated into five areas: Treatment room, ashing room, isotope preparation room, wash room, and a records area. Six years of experience with an outdoor cat colony led to this design. The design has proved itself during this year of use. No significant change in design is contemplated.

One hundred and eighty-six cats, of all ages and states of pregnancy were moved during one of two occasions. In early February, about 60 adolescent cats were shipped in cages in a heated van and arrived the next day in good condition. These cats were treated, just before shipment, either with prolipin, Vitamin C, or feline normal serum. No difference in disease incidence could be seen among these treated groups or between treated and untreated groups. The remainder of the animals traveled during mid-March by the same method. No immediate changes could be observed which could be directly related to the move itself. A series of complete blood counts were accomplished on ten 11-month old males from the first shipment. No significant change could be observed, for the first 60 days, in red cell, white cell, and differential parameters.

Since relocation, some generalized changes have occurred in the colony:

1) A lengthened anestrus period: General production has decreased with matings either not accomplished or non-productive. The production of the colony does not follow the previous production curve shown in Figure 7. The last litter was born in October; no others are expected to be born until possibly March; four full months (November-February) will contain no litters, even though this colony now contains about the highest population of breedable females since 1963. No cause for this 6 percent loss during this year is obvious.

A second production loss is caused by sterile matings experienced here. Fifty-one matings produced only 33 litters, a rate of only about 65 percent. This rate is the lowest this colony has experienced. Also, 707

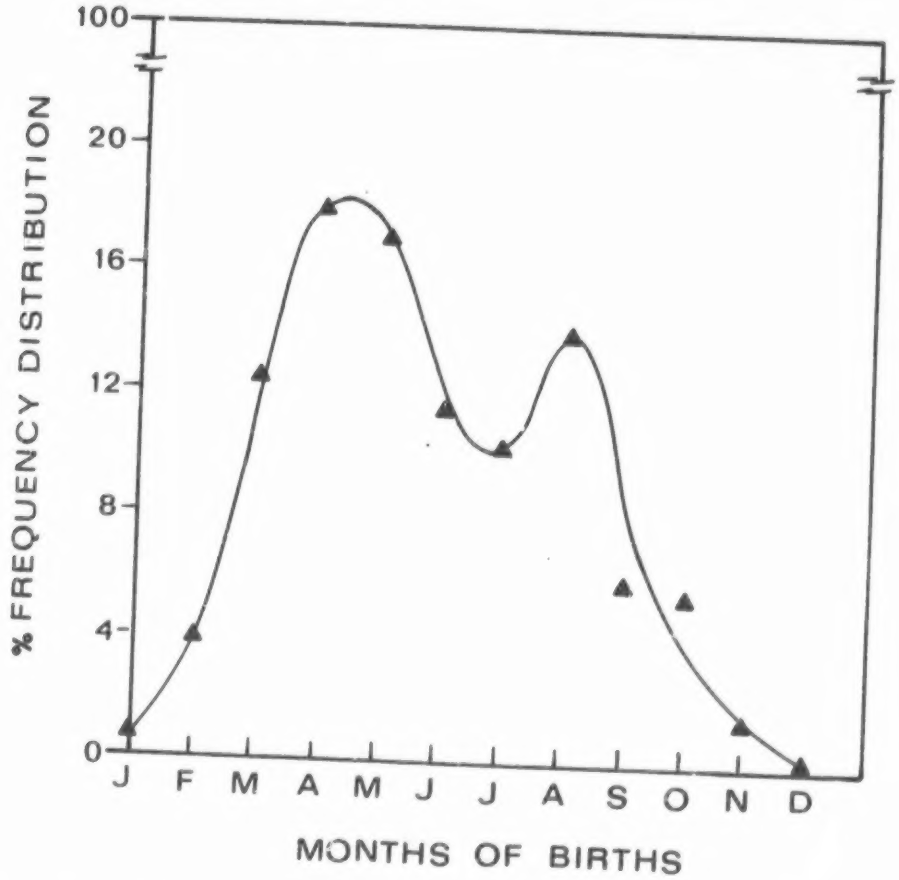

Figure 7. Monthly Distribution of 305 Litters Born from 1963 to 1969.

attempts to mate (placing an adult male with a queen in heat) were required to produce this conception rate. We have no obvious cause for the inefficient production, except that this may also be related to extreme weather conditions, or other factors.

We are trying other methods to stiumlate production. One holds considerable promise--induction of estrus by a series of injections of pregnant mare serum gonadotropin (PMSG), as per the technique of Emerson Colby of Dartmouth University. In November, four anestrus queens were given, each, 100 I.U. of PMSG, then 50 I.U. daily until they were in heat on the 8th or 9th day and then bred. Two of the four cats were later diagnosed pregnant by abdominal palpation. This method will be used as a production tool in a limited manner, to breed for non-experimental uses.

2) A series of clinical entities: Two abortions and two post-partum septicemias occurred with the cause unknown. The septicemias were fatal to dam and litters. The abortions occurred at 6-7 weeks post-breeding, and have not been experienced previously in the colony. An "epidemic" of submandibular abscesses in about 10 cats, which were located in the same or adjacent pens, were successfully treated. Cultured abscess material

yielded B-hemolytic streptococcus organisms. A group of young adults, mostly of the first group shipped here, evidenced chronic non-specific diarrhea, though otherwise normal. Minor changes in management procedures are proving successful in abating the condition.

At the moment, the colony contains 270 cats of all ages, an increase of 85 animals in 8 months of production. The 85 additional cats are the survivors of 200 cats added to the colony since relocation. A breakdown of causes for the losses follows:

Non-experimental losses	87
Experimental losses	40
Sent out of the colony	19
TOTAL	146

An unusual number of adult deaths were mainly due to septicemias and pneumonia-type diseases.

The colony is now mainly a production colony, with aims to reach a population of 500 to 550 cats by this time next year. Intensive breeding will be required, with expert management and professional care. Minor additions of genetics will be made both by acceptance of donation cats and by artificial insemination, using semen from cats kept out of the main colony in a small quarantine area, yet to be built. The facility is expected to grow with the population of animals to be housed in it.

As the blood and bone marrow are primary observation tissues for the ^{89}Sr effects study, we are characterizing these tissues. Normal values for blood are being developed for age and sex. Bone marrow and other tissue biopsy techniques are being developed, and we are developing a proficiency in reading marrow cell smears. An unusual familial chromosomal constitution has been noted in our line of Seal Point Siamese cats and is summarized on p. 196.

We are also engaged in growth and development studies, using weight, epiphyseal plate closure, blood formed element population and anthropomorphic measurements.

B. EFFECTS OF ^{89}Sr ON THE FELINE HEMATOPOIETIC SYSTEM

N. S. Nelson, E. Berman, R. G. Wolfangel

Daily doses of ^{85,89}Sr (1:100) were administered orally to three groups of cats, each consisting of two or three pregnant females, for 65 days, starting the day after breeding and ending the day before parturition. Each of the three groups of pregnant females received a different level of $^{85,\ 89}$Sr per day, one group receiving a 62.5 μCi/day dose, the second 93.75 μCi/day dose; and the third 125 μCi/day.

Of the eight cats bred, five did not conceive or implant their conception, as determined by abdominal palpation. This conception rate was about 70 percent of the mean conception rate experienced in non-experimental females during this same period. This reduced fertility may be related to a prior exposure to ^{89}Sr, but shows no correlation to the level of prior exposure.

Two of the three cats that produced litters lost the litters within 3 days. No. 776 cannibalized her litter, and No. 901 did not lactate, so the kittens died in spite of efforts to save them. No. 56 produced 4 kittens two of which were sacrificed--the other two are still alive.

Blood samples were taken from all queens prior to breeding and at about 7-day intervals thereafter until the end of the ^{85,89}Sr exposure period. The values reported for the first sample were used as reference values so that curves could be made for each group. The curves are expressed in percent of the initial value for ease in averaging the values. The initial values for individual animals are shown in Table 8 and curves of selected values in Figure 8.

TABLE 8. VALUES OF INDIVIDUAL CATS PRIOR TO BREEDING AND EXPOSURE

	Number per cm^3 of Blood					
Cat	RBC x 10^6	WBC x 10^3	Platelets x 10^3	Neutrophiles	Lymphocytes	MCHC
			62.5 μCi/day			
56	8.93	9.4	410	5264	3088	.35
917	7.90	14.7	390	8967	3822	.36
800	7.46	13.1	280	7074	3144	.35
			93.75 μCi/day			
776	7.93	24.4	510	14884	6344	.34
901	7.32	14.1	170	9306	3384	.36
1160	9.32	7.9	220	3476	3950	.35
			125 μCi/day			
40	9.07	19.5	550	12480	5460	.34
986	8.91	9.8	310	4606	4606	.35

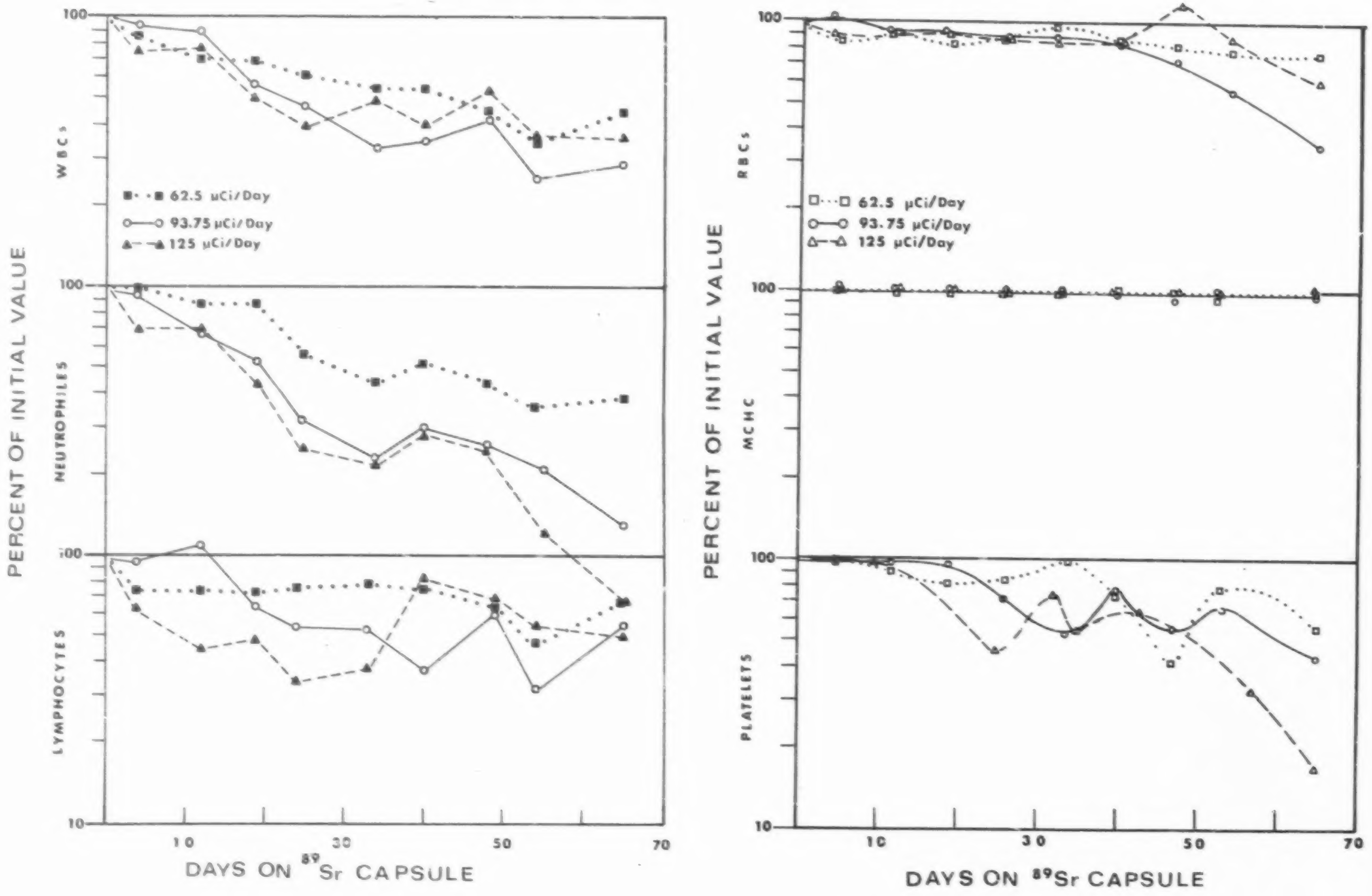

Figure 8. Percent of Initial Values of Various Blood Components from ^{89}Sr-Fed Cats.

The mean corpuscular hemoglobin concentration (MCHC) is unchanged during the exposure period, even though the red blood cell counts drop, especially near the end of the exposure. This suggests that the exposure causes a drop in RBC's by increasing the rate of destruction or decreasing the rate of replacement of RBC's rather than by influencing RBC size, shape or hemoglobin content. The constancy of MCHC indicates that the RBC's are morphologically normal.

The platelet count (Platelets) starts to drop by the 14th day after the start of exposure and continues to drop, even though there are abortive attempts to return to normal levels.

White blood cell counts drop continually almost from the day $^{85,89}Sr$ exposure starts. The component of the WBC that seems most affected is the granulocyte count. The curve of neutrophiles is shown since eosinophile count had dropped to about zero by the third week after dosing was started and remained at that level. Lymphocyte counts dropped initially, but then remained relatively stable.

The WBC responses might be interpreted in the following way: ^{89}Sr beta radiation from strontium deposited in bone has nearly completely destroyed the capability of the bone marrow to produce platelets and granulocytes. This is shown by the continually decreasing counts of these cells in peripheral blood.

The lymphocyte response shows a similar pattern if evaluated using Loutit's,[1] two lymphocyte populations (1) a short-lived lymphocyte produced in the marrow and (2) a long-lived lymphocyte of extramedullary origin. The initial drop in lymphocyte count is due to the short-lived population. Destruction of marrow progenitors prevents the population from recovering to normal levels. The lymphocyte population remains stable after the short-lived members disappear because either the long-lived lymphocytes are not affected, or, since the origin is extramedullary, the injured members of the population can be replaced.

Figure 9 shows the pattern of hematologic changes in cat No. 56 and her two kittens. The values for No. 56 are a continuation of the response shown in Figure 8.

In the kittens MCHC starts higher than normal, but drops to normal within 3 weeks; RBC start a little low, but become normal in seven weeks. The kittens' platelet counts were normal, but the WBC's showed a peak shortly before weaning (day 55). This peak was due to an increase in the number of neutrophiles and probably reflects an infection of subclinical nature. The lymphocyte count was approximately normal.

Cat No. 56 was sacrificed on 9 December 1969 at which time her platelet count was zero and had been zero since 26 November 1969: WBC was 2.8×10^3 cells/cm^3. This animal had a severe hemorrhagic diathesis and was sacrificed because hemorrhage could not be controlled. She was sacrificed 180 days after she received the first dose of $^{85,89}Sr$.

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[1] Loutit, J.F. Haematological Effects of Radiation, p. 39-47. In Guide-Lines to Radiological Health, McGill University, Montreal, August 1967.

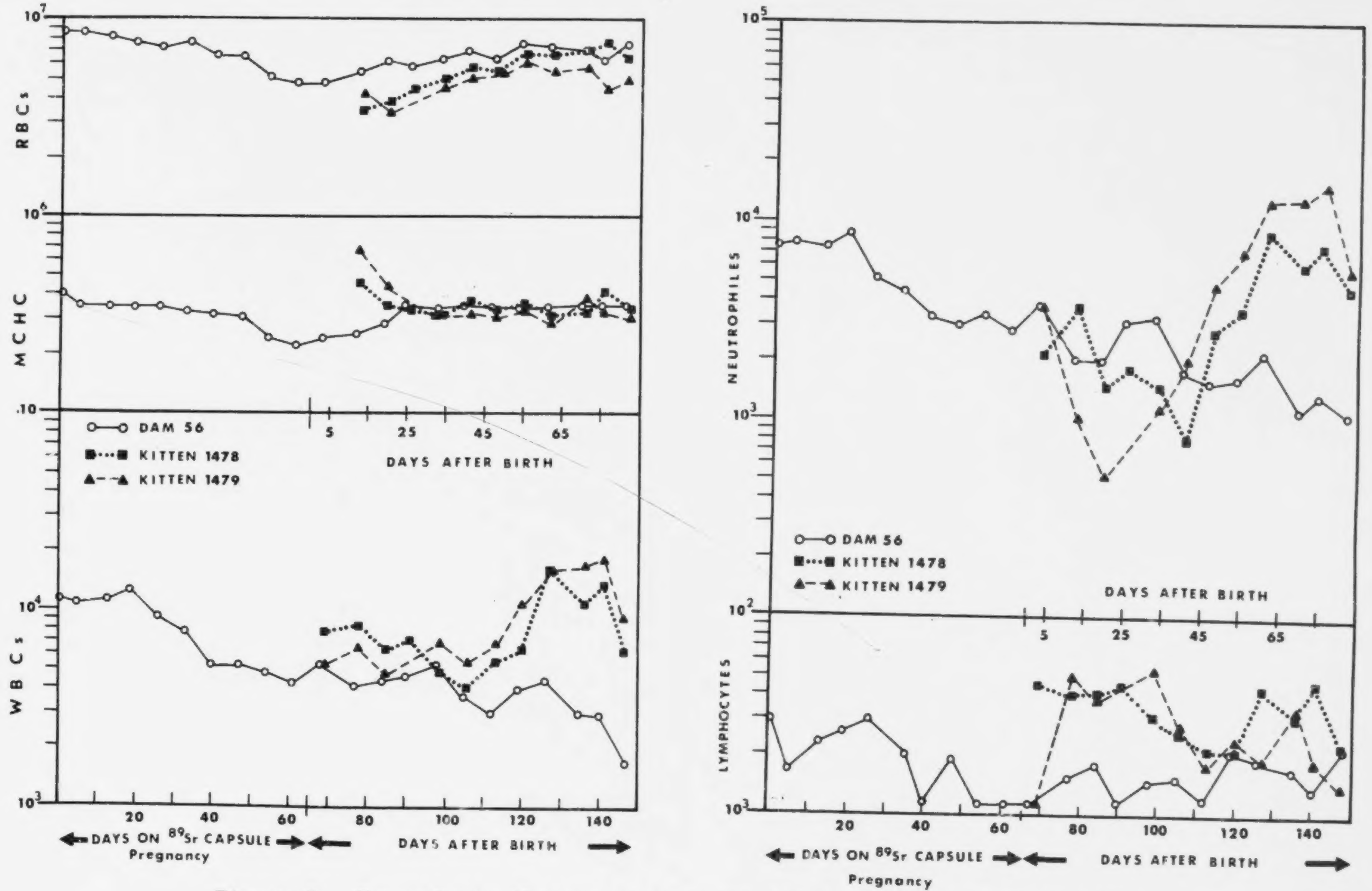

Figure 9. Hematologic Changes in Dam and Kittens Before and After Birth.

C. INCREASE IN BODY BURDEN IN KITTENS NOT FOSTERED TO A "COLD" DAM AFTER IN UTERO EXPOSURE TO STRONTIUM 85

N. S. Nelson, E. Berman

The question of body burden in kittens after an in utero exposure to ^{85}Sr was investigated in 3 litters. Previous experiments in fostering of kittens have shown that the mortality rate in fostered kittens may approach 70 percent during the first 3 months after birth.

In some experimental designs, the need for controlling the neonate body burden, and thereby the rad dose, has become an important factor. Although there is some data on the rate of increase of body burden in kittens born of and nursing on a dam which is receiving daily doses of ^{85}Sr, there are no data for the case where radioisotope administration is stopped 24 hours prior to the birth of the kittens. Since fostering of neonate kittens to cold dams as a means of controlling neonate body burden incurrs such high postnatal reproductive wastage, a pilot study was made to determine the effect of halting the dam's ^{85}Sr dose 24 hours before parturition.

In this pilot experiment, 3 female cats were bred. Each received 0.5 µCi/day of ^{85}Sr starting the day after breeding and continuing until 24 hours before the birth of the kittens. A special vest was placed on each queen, at the time parturition was expected, to prevent the kittens from nursing until a 0 day whole-body burden could be determined. All whole-body counts were made 12 inches from a 4 x 4 inch NaI (Tl) crystal in a steel cave.

Figure 10 shows the whole-body curves for the mean value of each litter. The curves increase from day 0 (birth) until about day 25. The peak body burden reached was from 140 percent to 190 percent of the body burden at birth. The peak coming at day 25 is coincident with the time (about day 21) when the kittens first started eating solid food.

Since the sole source of this additional ^{85}Sr is maternal milk, and the amount of ^{85}Sr in the milk may be reduced by feeding the dam a high calcium or high strontium diet, or adding alginate to the diet immediately after birth, it should be possible to reduce or eliminate this increase in the body burden of the nursing kittens. Additional experiments are under way to determine the effect of such treatments to the dam.

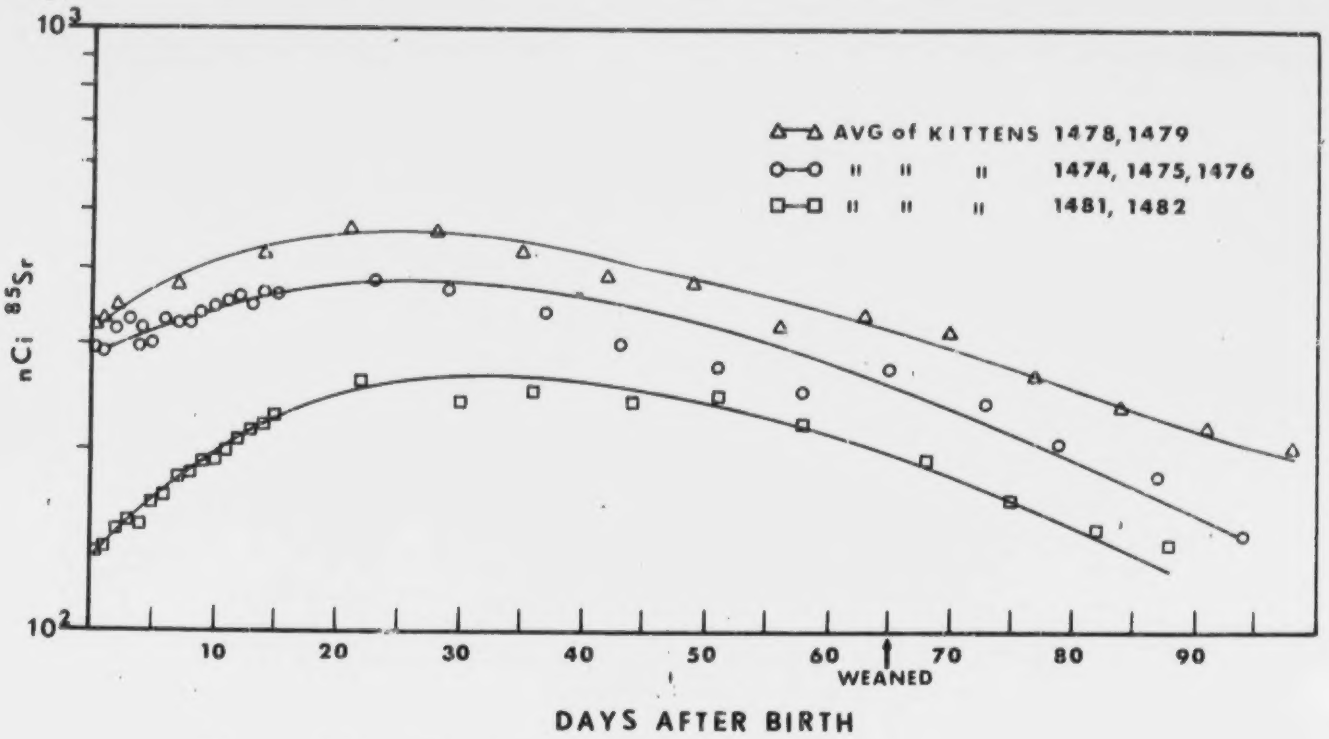

Figure 10. Body Burdens of Kittens Exposed In Utero.

D. BODY BURDENS PRODUCED IN CATS BY DRENCHING

N. S. Nelson

Most of the chronic administration of radiostrontium to felines in this laboratory has been by pill. Pilling has always been the most convenient and accurate method of chronic dosing for most of the isotopes used. However, recent experiments using high levels of ^{89}Sr have produced a local hazard to the fingers of the operator due to high beta levels. Both pilling forceps and balling guns have been used successfully to avoid high beta doses, but they are not as convenient as fingers, and the forceps particularly can injure the animals' soft palate.

To avoid the high beta dose through the thin capsule walls or, alternatively, injury to experimental animals, some animals were dosed by drench. Two litters of three neonate kittens each and one litter of three adolescent (9 month) cats were given a daily dose of 0.5 μCi ^{85}Sr for 65 days. The dose was given as a drench of 50 to 150 μl of $^{85}SrCl_2$ solution. Initial dosing caused heavy salivation, but when the $^{85}SrCl_2$ solution was diluted for dosing, using tap water rather than 0.5 N HCl, the salivation stopped.

Uptake curves are shown in Figure 11. The curve of the mean for the six kittens was starting to plateau by the end of the dose period with an individual body burden about 40 times the daily dose. The curve of the mean for the three adolescent cats shows a similar pattern, but the final body burden in this case is about 14 times the daily dose. This is comparable to the 15 times the daily dose burden observed in similar age experimental animals dosed by pill in Cincinnati.

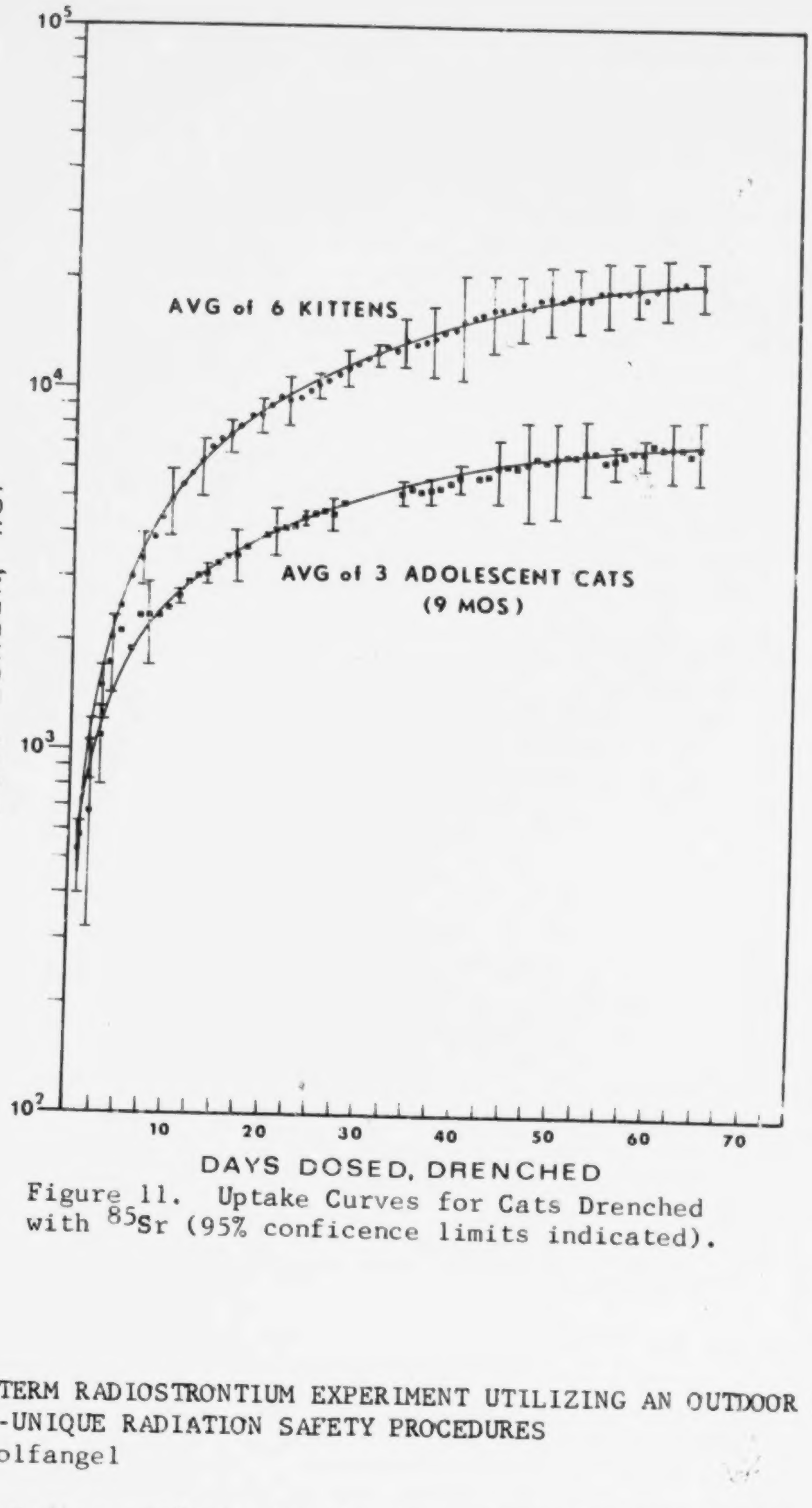

Figure 11. Uptake Curves for Cats Drenched with ^{85}Sr (95% conficence limits indicated).

E. A LONG-TERM RADIOSTRONTIUM EXPERIMENT UTILIZING AN OUTDOOR FELINE COLONY--UNIQUE RADIATION SAFETY PROCEDURES

R. G. Wolfangel

In the studies conducted by the Toxicologic Studies Section, as many as 40-50 cats in the outdoor feline colony will simultaneously receive 65 consecutive daily doses of ^{89}Sr, each cat's dose ranging upwards to 100 μCi. Maintenance of the experimental cats in an outdoor colony calls for implementation of rigid health physics regulations, handling procedures, and special laboratory design, to maximize safety and minimize personnel exposure.

Laboratory Design

The Toxicologic Studies Section is functionally and physically divided into a main laboratory and the cat facility. The main laboratory contains office space, autoradiography, microscopy, counting, dissection, serology, chemistry, surgery, and necropsy laboratories. Except for the counting and surgery labs, only sacrificed animals or specimens thereof are processed in the main laboratory to reduce the risk of personnel exposure.

Special attention has been given to the dissection lab. A "hot" cat is not released for dissection until the following limits are attained by survey: 2.5 mR/hr at 1 ft from the cat, and 10 mR/hr at 1 cm. In addition, TLD dosimeters are abundantly employed at various hand, wrist, arm, and body sites of the dissectionists in order to obtain dosimetry records and determine the adequacy of the prescribed limits.

The design of the cat facility proper is depicted in Figure 12. Entry into the colony is restricted and the entire complex is surrounded by a security fence. Run 1 is a single row of 20 adjacent pens, each 4 x 12 feet; the rear of each pen is connected to a drainage trough so that all liquid waste generated within these pens can be collected in the plumbing network. It is in this run where the cats will be maintained during dosing with ^{89}Sr. Each pen contains a galvanized tray (2 x 3 x 2/3 feet) filled to a depth of 2 - 3 inches with "Kitty Litter." Owing to the general cleanliness of the cat, almost without exception, each will excrete in the litter and then cover the excreta. Thus, the vast majority of radioactive strontium contained will be found in two places: (1) in the cat, (2) in the Kitty Litter. Secondly, the cat metabolizes ingested strontium in such a way that 100 times more Sr is excreted in the feces than in the urine. This fact further simplifies the radioactivity control since the simple act of sifting the Kitty Litter, using a specially designed rake-type shovel to pick up the dried fecal clumps, removes more than 90 percent of the ^{89}Sr contained in the litter boxes. The fecal material is then transferred to 50 gallon steel barrels for storage, then shipped for disposal. In addition, the Kitty Litter is monitored on a regular basis; when residual radioactivity reaches 25 mR/hr at 1 ft, the Kitty Litter is transferred to the waste barrels and replaced with new.

The floor and house of the pens are checked weekly by taking smears, when removable radioactivity of 2500 dpm/dm^2 is found, the entire pen is hosed down and scribbed, discharging all waste water into the collection tanks. (In colder weather, the pens are dry scraped and then vacuumed.)

The work area receives the same attention as do the pens. The entire run has been designated a foot cover area, and personnel must wear protective foot covers, laboratory coats, and gloves upon entering.

As previously mentioned, all waste water originating in run 1 is retained in 1 of 4 underground collection tanks. Waste from runs 2 and 3 can be discharged directly to outside sewage lines, or retained in the

FP - Food Preparation
TA - Treatment Area
RS - Radioisotope storage and preparation room
AR - Ashing room
MW - Miscellaneous work and storage area

T1 - Waste tank
T2 - Waste tank
T3 - Waste tank
M-1 - Monitoring tank
X - Control valve

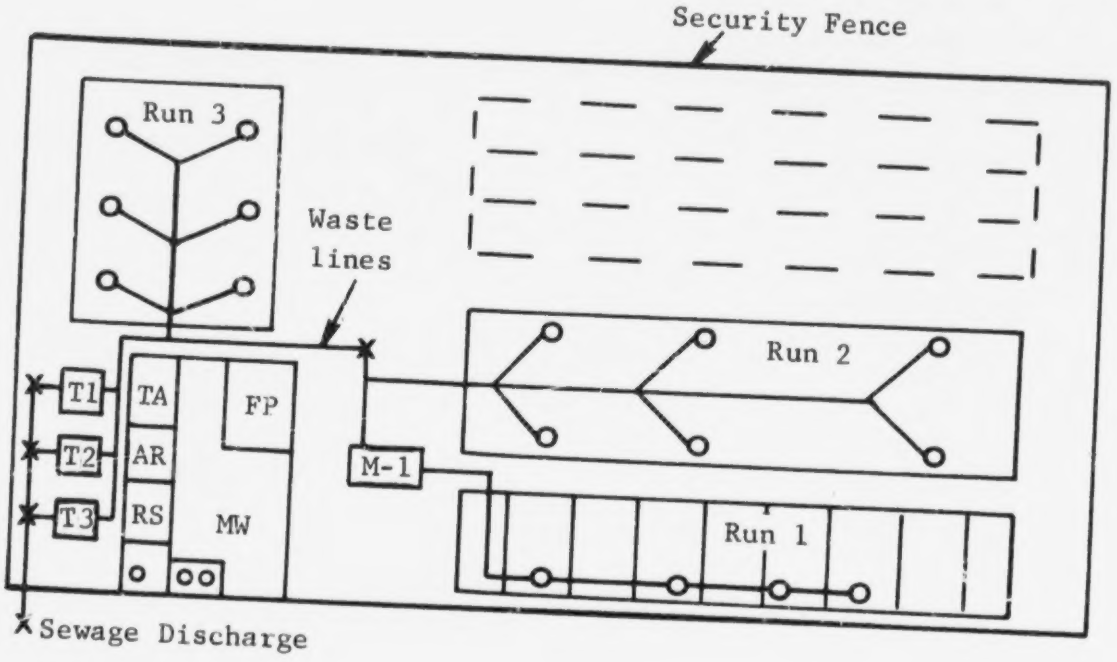

Figure 12. Diagram of Cat Colony Facility.

collection tanks. Each of the 4 tanks has a liquid level indicator which is checked daily. As one tank is filled, it is closed, and liquids diverted to one of the other tanks. Then samples are removed from the full tank and checked for radioactivity. The release or no-release decision is made on the basis of the radionuclide with the lowest MPC. Since our laboratory is intending to use only ^{85}Sr and ^{89}Sr, the most critical radionuclide appears to be ^{90}Sr, which is a contaminant in our ^{89}Sr as supplied by Oak Ridge National Laboratory up to levels of 10 percent.

Radionuclide Administration

Immediately prior to dosing the cats, they are collected from their respective runs and placed in a stainless steel cage and the cage rolled to the counting room of the main laboratory where cats are monitored for whole-body radioactivity levels. After counting, they are returned to the colony building where they are given their radioisotope. Two people are required to administer the radiostrontium. One restrains the animal, the second prepares the syringe, then expels the liquid directly into the animal's mouth, a technique referred to as drenching. The cats are then returned to their respective pens where they remain relatively undisturbed until the following day when they go through the identical procedure.

The drenching operation requires the most care and presents the greatest likelihood of contaminating personnel. Each person involved in the drenching is required to wear protective clothing. A list of radiation safety rules, as proposed by the joint effort of the Bureau's Radiation Safety Officers has been adopted expressly for use in the Toxicologic Studies Section.

F. ^{90}SR DOSE-RATE RESPONSES IN YOUNG AND ADULT CATS: EVALUATION BY ISODENSITOMETRY

L. S. Rosenstein

When an animal is exposed to ^{90}Sr, a bone-seeking radioisotope, the deposition pattern in bone is qualitatively similar to that of calcium, the ion normally laid down in skeletal growth.

The experiment to be discussed was designed to quantitate absorbed dose rates and their regional location in bone as a function of increasing age, using radioautography and isodensitometry.

All animals in the study were born from radioactive dams and exposed to ^{90}Sr *in utero* and during nursing. After 25 to 30 days of age, the radioisotope was administered daily, ad libitum in the food. Animals were sacrificed by exsanguination at 50 (weanling), and 500 (adult) days of age. Quantitative radioautography was performed on selected bones. The bone samples were cut into infinitely thick segments and embedded in Bioplastic. They then were apposed to Kodal NTB or NTB2 nuclear track plates for the required radioautographic exposure time. The following radioautograms were analyzed by isodensitometry[1] using the radioautographic dosimetry method of Marshall, et al.[2]

1) Radioautograms of the infinitely thick longitudinal proximal and distal segments of femur, humerus, tibia, and vertebra.
2) Radioautograms of the infinitely thick transverse segments of femur, humerus, tibia, radius, ulna, and rib.

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[1] Miller, C.S. Isodensitometry as an Aid in the Analysis of Photographically Recorded Information. Preprint No. 101-89. Presented at the 101st Technical Conference of the Society of Motion Picture and Television Engineers, April 16-21, 1967.

[2] Marshall, J.H. et al. Radiation Res., 10:213, 1959.

Isodensitometry was performed on the isodensitracer (Technical Operations Incorporated, Burlington, Massachusetts). This is a high speed, direct reading isophotometer that produces a two-dimensional, coded printout or contour plot. The basic components of the isodensitracer are: 1) a Joyce Loebl Dual Beam Microdensitometer (Joyce Loebl and Company, Ltd., Princesway, Team Valley, Gatehead 11, England); 2) a programmer; 3) automatic specimen table; 4) automatic recording table; and 5) Isophot recording pens.

Photometric analysis is accomplished using a single tungsten light source and channeling the light along two paths. One path goes to and through the sample and then to a photomultiplier. The other goes to and through an optical attenuator (Gray wedge-optical glass plates composed of density gradient) and then to the same photomultiplier as above. The photomultiplier alternately analyzes each beam by means of an electronic beam chopper (3600 CPS). The instrument operates on the principle of optical null balance achieved by movement of the gray wedge optical attenuator. When nulling is achieved, a symbol as part of a specific program is printed out.

Programing is made possible by a 64 pad linear encoder and a moveable gray wedge optical attenuator used in combination with a fixed 64 contact linear commutator. The encoder commutator and the gray wedge optical attenuator control the write-out mode of the isophot pen according to the density changes in the specimen.

The type of programing used in the isodensitracer is different from that of conventional densitometers. The isodensitracer writeout technique is called the dropline method which shows density increments as either dots, lines, or spaces. Density increments using the dropline method can be recognized by the progression of dots, lines, or spaces. For example, increasing density may be noted as the progression of spaces, dots, and lines of a specific color. Decreasing density appears as lines, dots and spaces. Programing is a continuous, cyclic process, and can be repeated until all encoder positions are used. The density increments are preset and thus represent a fixed fraction of the useful range of the optical attenuator.

The results of this study are summarized in Tables 9 and 10. The data shown for the epiphysis, metaphysis, and diaphysis of the long bones represent maximum responses. Maximum dose rates are also given for the transverse shaft as well as the rib and vertebra. The dose rates shown for the marrow cavity in the transverse segment and the longitudinal segments of the long bones represent dose rates observed in the central part of the cavity.

TABLE 9. SUMMARY OF DOSE RATE RESPONSES IN 50-DAY-OLD WEANLING CATS[a]

Bone Segment	Location	Response (Rads/day)
Proximal Femur	Plate-head	2.28
	Plate-G.Trochanter	1.71
	Epiphysis	2.47
	Metaphysis	2.47
	Shaft	2.28
	Marrow cavity	0.95
Distal Femur	Plate	2.28
	Epiphysis	2.47
	Metaphysis	2.47
	Shaft	2.28
	Marrow cavity	0.76
Transverse Femur	Shaft	1.26
	Marrow cavity	0.50
Proximal Humerus	Plate	2.56
	Epiphysis	2.96
	Metaphysis	2.96
	Shaft	2.36
	Marrow cavity	1.18
Distal Humerus	Plate	2.14
	Epiphysis	2.49
	Metaphysis	2.49
	Shaft	2.31
	Marrow cavity	1.25
Proximal Tibia	Plate	2.14
	Epiphysis	2.14
	Metaphysis	2.49
	Shaft	1.96
	Marrow cavity	1.42
Distal Tibia	Plate	2.14
	Epiphysis	1.96
	Metaphysis	2.14
	Shaft	1.78
	Marrow cavity	0.92
Transverse Tibia	Shaft	1.49
	Marrow Cavity	0.79
Transverse Radius	Shaft	1.36
	Marrow cavity	1.12
Transverse Ulna	Shaft	2.21
	Marrow cavity	1.01
Transverse Rib	Center	1.36
Vertebra	Body	1.66

TABLE 10. SUMMARY OF DOSE RATE RESPONSES IN 500-DAY-OLD ADULT CATS

Bone Segment	Location	Response (Rads/day)
Proximal Femur	Epiphysis	1.47
	Metaphysis	1.74
	Diaphysis	1.20
	Marrow cavity	0.20
Distal Femur	Epiphysis	1.44
	Metaphysis	2.58
	Diaphysis	1.23
	Marrow cavity	0.41
Transverse Femur	Diaphysis	1.00
	Marrow cavity	0.25
Proximal Humerus	Epiphysis	1.59
	Metaphysis	2.30
	Diaphysis	1.62
	Marrow cavity	0.53
Distal Humerus	Epiphysis	1.32
	Metaphysis	0.88/1.49
	Marrow cavity	0.53
Transverse Humerus	Diaphysis	1.37
	Marrow cavity	0.29
Proximal Tibia	Epiphysis	1.70
	Metaphysis	2.44
	Diaphysis	1.69
	Marrow cavity	0.47
Distal Tibia	Epiphysis	1.41
	Metaphysis	1.93
	Diaphysis	1.32
	Marrow cavity	0.53
Transverse Tibia	Shaft	1.20
	Marrow cavity	0.32
Transverse Radius	Shaft	1.81
	Marrow cavity	0.69
Transverse Ulna	Shaft	1.60
	Marrow cavity	0.80
Transverse Rib	Cortex	0.83
Longitudinal Vertebra	Trabecular Bone	2.08

G. PATHOLOGIC EFFECTS OF CONTINUOUS STRONTIUM-89 INGESTION IN MICE[1]

James F. Wright

This experiment was designed to test the significance of dose rate and age on the induction of hematopoietic neoplasms by continuous feeding of ^{89}Sr to mice for 180 days. Effects were compared among three inbred strains of mice first exposed at conception or at weaning. Over 1,500 mice were utilized, as well as a smaller number of adults apart from the main experiment.

There was no apparent radiation effect on litter size or on survival to 6 months of age. Evaluation of the data to 14 months of age showed marked differences in survival rate and cumulative incidence of hematopoietic or bone tumors. The RFM strain had a high incidence of hematopoietic neoplasms beginning at 6 months of age; the BALB/c and C57BL strains had negligible hematopoietic neoplasms but began developing bone tumors at 10 months of age. Of 11 tumors in C57BL mice, 3 were in the pelvic region; BALB/c mice had no pelvic tumors but a high number (18/21) of hind limb tumors; no RFM mice developed bone tumors to 14 months of age. Female mice of all strains had higher tumor incidence and earlier mortality than male mice.

The dose-rate effect was evident. The highest-level ("7-level") groups (60 μCi ^{89}Sr/g dietary Ca daily) had approximately twice as many hematopoietic neoplasms as did the "6-level" groups (20 μCi ^{89}Sr/g dietary Ca daily); there were no bone tumors in control animals, two in 6-level animals, and 30 in 7-level animals. The survival rate for 7-level mice was about one-half the control survival rate and two-thirds to one-half the 6-level survival rate to 14 months of age. However, the number of offspring weaned in the 7-level RFM and BALB/c mice at least equalled that of the controls. No clear difference in survival rate or tumor incidence is yet apparent between animals first exposed at gestation and those first exposed at weaning.

The cumulative rad dose to 14 months was 4,800 rads at the 7-level for animals first exposed during gestation, and 3,900 rads for 7-level mice started at weaning. These animals will be studied for the major part of their lifespan.

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[1] Work performed at Radiobiology Laboratory, University of California, Davis, under Contract AT(04-3)472 for the U.S. Atomic Energy Commission.

PATHOLOGIC STUDIES SECTION

Dr. C. David Lytle, Chief[1]

The Pathologic Studies Section has a continuing research program which investigates the nature and extent of the interaction between radiation and synergistic or co-insultive agents such as chemicals and viruses in the induction of cancer or in the production of other radiation-related effects.

The past year the Section has shown marked progress in studies relating to radiation activation of virus in cultured cells (see p. Irradiation of cultured 3T3 cells resulted in the possible activation of repressed viruses in the cells which transformed the cells and led to tumor production following injection of the transformed cells into the host.

The results of these investigations may provide a model for studying radiation-induced mechanisms that lead to cancer in humans. Considerable extension of these studies will be required before any final conclusions can be drawn about the relationship between cancer induction, radiation exposure, and viral transformation.

Other studies in the Section emphasize relationships such as the interaction between radiation and known environmental toxic agents like aflatoxin. Success in establishing lines of cultured cells from a radiation-induced mammary tumor may lead to information about the unique susceptibility of the rat to mammary tumor development following radiation, as well as possible etiologic agents. Broadening program interest into microwave effects is reflected in the preliminary report on whole-body cooling and other studies involving microwave radiation.

A. PROLONGATION OF LIFE IN A MICROWAVE FIELD BY MEANS OF AN ENVIRONMENTAL CHAMBER

Lawrence R. Muroff, George Samaras

A 200 gram Osborne-Mendel rat exposed in a microwave field to a power density of approximately 100 mW/cm^2 at 2450 MHz, will die in approximately 14 minutes. Survival time can be extended by cooling the rat during the microwave exposure by means of external forced convection to that his rectal temperature remains constant at slightly above its normal value throughout the experiment.

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[1] Dr. Lytle is the current Chief of the Section; Dr. B.C. Ward was Chief until August 1969; Dr. N.C. Telles was Acting Chief, August-December 1969.

As indicated in Figure 13, a small mammal can be restrained in a microwave field while its immediate environment is flushed with compressed air, pre-cooled by liquid nitrogen. Rectal temperature is constantly monitored by means of a rectal thermistor placed parallel to the field's Poynting vector so as to avoid inducing local "hot-spots." The animal's pulse can be monitored with a pressure cuff placed on its tail.

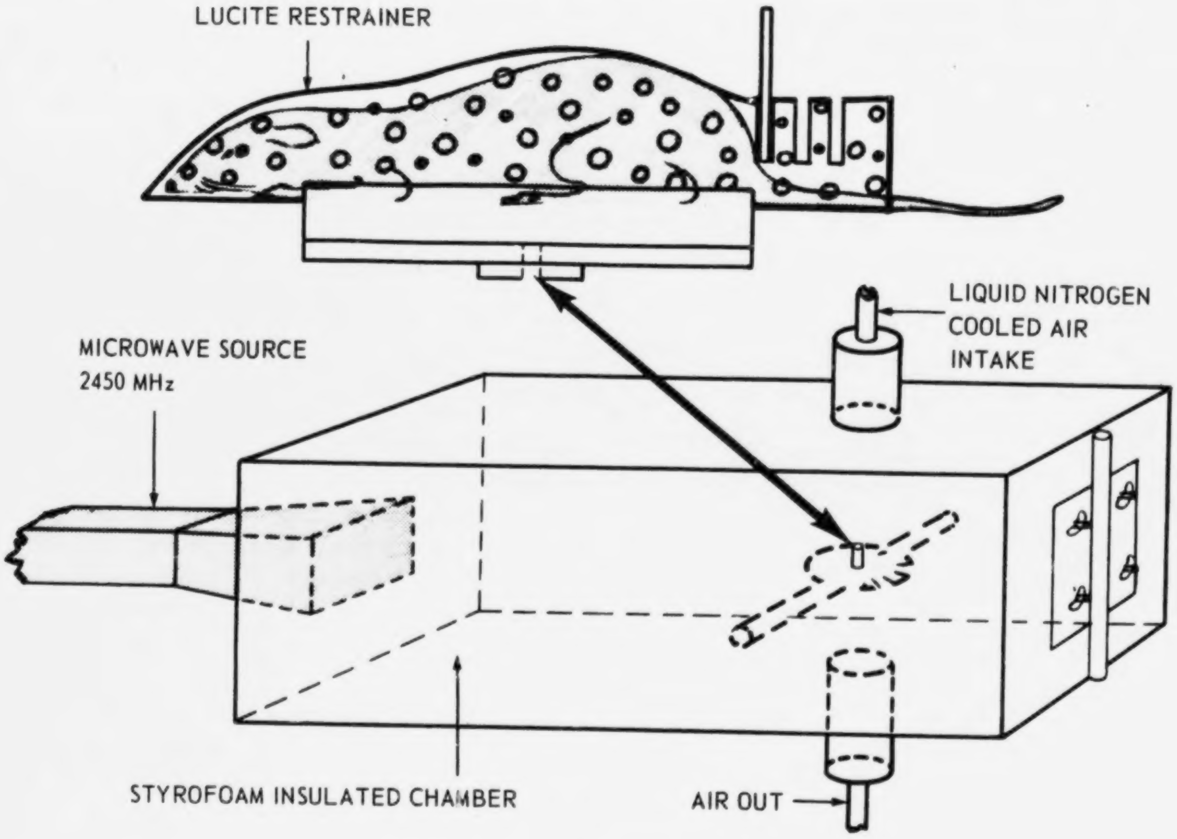
LUCITE RESTRAINER
LIQUID NITROGEN COOLED AIR INTAKE
MICROWAVE SOURCE 2450 MHz
STYROFOAM INSULATED CHAMBER
AIR OUT

Figure 13. Environmental Chamber Used to Cool Laboratory Rodents.

In a pilot project two Osborne-Mendel rats were exposed separately to a microwave field (100 mW/cm^2, 2450 MHz) while confined in the environmental chamber. Rectal temperature was maintained at 39°C. Each rat was maintained in the field for approximately one hour. The rats appeared (grossly) normal at the end of the exposure, and were returned to their cages for observation. Over a 30-day period following irradiation the rats seemed (grossly) normal. All findings at autopsy after the observation period were within normal limits as were the microscopic examinations of necropsy specimens of lung, ovary, eye, brain, liver, kidney, thyroid, and adrenal gland.

An expanded experiment is currently in progress.

B. CHARACTERIZATION OF RAT MAMMARY TUMOR IN VIVO AND IN VITRO.

K. B. Hellman

Mammary tumors in the rat may develop spontaneously as well as by induction with X irradiation and/or chemical carcinogens. Many studies have been done on the whole animal. In addition, studies have more recently been focused at the cellular level. Furthermore, the presence of virus-like particles in certain mammary tumors has led to studies attempting to demonstrate the relationship between virus and tumor.

In view of these more recent reports, investigations have been conducted dealing with two primary objectives: (1) to establish and characterize the growth of rat mammary tumor cells in vitro, and (2) to investigate the capacity of rat mammary tumor cells grown in vitro to initiate tumor development in Osborne-Mendel rats. In conjunction with these studies, a search was made for the presence of viruses which may be associated with the mammary tumor.

As a result of our efforts, rat mammary tumor cells have been established to grow in vitro and are presently being carried in the laboratory as a permanent cell culture; they have undergone 42 in vitro serial transfers, to date. These cells were obtained initially from a papillary cystadenoma which developed in a female Osborne-Mendel rat 6 months after being X irradiated with 450 R to the whole body, the animal being two months old at that time.

Early cell cultures (1-3 serial transfers) were morphologically similar to the original tumor, consisting primarily of epithelial cells with acini formation. At the third serial transfer some cultures were scraped with rubber policemen when harvesting while others were trypsinized with 0.25 percent trypsin. Morphological differences began to appear at the ninth transfer, with certain cultures becoming fibroblastic. The morphology of the cultures seemed to stabilize at the ninth in vitro transfer and has remained constant with the result that three morphologically distinct cell cultures are now being carried.

These are:

RMT-A - harvested by scraping with rubber policemen, epithelial in structure, acini appear at 7-10 days in culture.

RMT-C - harvested with 0.25 percent trypsin, epithelial (cuboidal) in structure, acini appear as in RMT-A.

RMT-D - harvested with 0.25 percent trypsin, fibroblastic in structure, acini never appear.

Attempts were made to correlate the differences in morphology between RMT-A and RMT-D cell cultures with differences either in the number or structure of their chromosomes. Preliminary studies indicate no difference in the numbers of chromosomes in either RMT-A or RMT-D when compared with the karyotypes of the normal rat; both cell cultures possess a diploid number ($2n = 42$) of chromosomes. However, a somewhat longer number three chromosome has been observed in both RMT-A and RMT-D when compared to the normal. The significance of this observation has not been determined. More cells must be analyzed before any definite conclusions can be reached.

Since a model in vitro - in vivo cell system would be valuable in many studies, it was of interest to determine the capacity of rat mammary tumor cells grown in vitro to initiate tumors in the host of origin. Therefore, cell cultures carried in vitro for 7, 18, and 37 passages were inoculated into 3-week-old female Osborne-Mendel rats.

Although a small number of animals were used in the preliminary pilot experiments (4-6 animals per group) and the more recent experiment using approximately 20 animals per group has not been completed, certain interesting observations have been made.

(1) RMT cells grown in vitro are capable of inducing tumors in all (100 percent) irradiated (350 R, whole body) and non-irradiated animals inoculated.
(2) Tumors appear in all inoculated animals at seven days. Early cell cultures give rise to tumors in non-irradiated animals which grow to 4-5 cm in diameter before regressing at 14 to 17 days; those tumors developing in irradiated and inoculated animals grow to 5.5 to 7 cm in diameter and may persist from 31 to 84 days.
(3) Animals inoculated with RMT-D cells develop tumors which regress at a slower rate than those of animals inoculated with RMT-A cells. The following observation was noted when the 35th in vitro passage cell cultures were used:

Days after initial tumor development	Percent Regression	
	RMT-A	RMT-D
12	19/24 - 79%	9/21 - 42.8%
31	24/24 - 100%	19/21 - 90%; tumor still present in two animals

(4) Early cell cultures (7 passages) gave rise to papillary cystadenomas, whereas 18th passage RMT-D cultures initiated fibroadenomas. Results of the most recent experiment are not complete at this time. In all cases, the tumor remained localized at the site of cell inoculation and did not metastasize.
(5) These results may indicate functional differences which may be correlated to the structural differences observed between RMT-A and RMT-D cells. However, additional experiments must be conducted to further substantiate these findings.

Attempts to implicate a virus either as the etiological agent or as being associated with the tumor have to date been unsuccessful. Electron microscopic observations have been made of normal rat mammary tissue, RMT-A and RMT-D cells, and tissue obtained from a tumor induced by inoculation with RMT cells. In a preliminary study, using frozen and thawed nonviable RMT cells as inoculum, a fibroma was induced in one irradiated animal. The reason for this is not known. Although all cells appeared to be nonviable microscopically, a few viable cells could have persisted which could give rise to a tumor. These studies will be repeated using other methods for preparing the cell extract.

The capacity of RMT cells to initiate tumor formation in vivo, when inoculated into the Osborne-Mendel rat, the host of origin, presents a convenient method for obtaining large quantities of tissue for certain biochemical studies and for investigations of similarities and differences between cells growing in the in vivo and the in vitro states.

C. EFFECTS OF WHOLE-BODY RADIATION AND EXPOSURE TO EXTERNALIZED MAMMARY TISSUE ON RAT MAMMARY CARCINOGENESIS

Billy C. Ward, Joe R. Childress, Norman C. Telles

Previous investigation of radiation-induced mammary tumors has indicated that systemic agents, such as 3-methylcholanthrene[1] and ethionine[2], cause an additive effect in mammary tumor incidence when these agents are used in conjunction with radiation. Polycyclic hydrocarbons, such as 3-methylcholanthrene and 1,2 - 5,6 dibenzanthracene, have been shown to inhibit the immune response.[3] One explanation for the success of a carcinogenic process would be by inhibition of immunologically competent cells.[4] The purpose of this experiment was to determine if the increased incidence of mammary tumors in rats exposed to whole-body radiation was a direct effect of X rays upon mammary tissue or partially the result of such systemic factors as the suppression of immune mechanisms.

Methods and Materials

A total of 146 female Osborne-Mendel rats was assigned to one of 4 treatment groups in an unselected manner (Table 11). All rats were 2 months of age at treatment.

TABLE 11. NUMBER OF MAMMARY TUMORS IN IRRADIATED RATS

Treatment	Rats in Treatment Group	Mammary Tumors (R) ING[a]	Mammary Tumors (L) ING[b]	Total Mammary Tumors
Controls	34	1	1	5
Flap only	36	3	2	7
WB + F[c]	38	6	4	14
WB - F[d]	38	4	1	11

a. Right inguinal area.
b. Left inguinal area.
c. Whole-body exposure = 100 rads; flap exposure = 400 rads.
d. Whole-body exposure = 100 rads; flap not exposed.

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[1] C. J. Shellabarger, J. Natl. Cancer Inst. 38:73-77 (1967).

[2] N. C. Telles and B. C. Ward, Rad. Res. 37: 577-589 (1969).

[3] M. C. Brenbaum, Brit. Med. Bull. 20: 159-164 (1964).

[4] Mac Farlane Burnet, Brit. Med. Bull. 20: 154-158 (1964).

A flap of mammary tissue at the right inguinal area was surgically externalized, but not detached from the body, in all four treatment groups (Figure 14.) The animals in the "flap only" group were given 400 rads to the externalized flap, and they received no whole-body exposure. The animals in the WB+F group were given 400 rads to the flap and an additional 100 rads whole-body dose. A 3-cm diameter steel cone collimator was employed for flap exposure. The animals in the WB-F group were given 100 rads whole-body exposure. In all cases during whole-body exposure, the flap was shielded with 3 mm of lead.

Radiation was delivered by an X-ray machine operated at 250 kVp. For flap exposure, no filtration was added; however for whole-body exposure, 1 mm Cu and 1 mm Al filtration was used. The target-to-rat distance was 50.7 cm. The time for flap exposure was 2.3 minutes for approximately 400 rads. The time for whole-body exposure was 1.6 minutes for approximately 100 rads. All animals were anesthetized with sodium pentothal.

The animals were housed two to a cage in a facility with controlled temperature and humidity. All animals were supplied with Purina rat chow and water ad libitum. Chlorotetracyline (200 mg /gal) was added to the drinking water to reduce deaths from respiratory diseases. The animals were checked monthly for mammary tumors and daily for deaths over a period of 18 months. Autopsies were performed on all dead animals and tissues were taken in all cases for histological examination. Tissues were fixed in 10% neutral, buffered formalin and stained with hematoxylin-eosin.

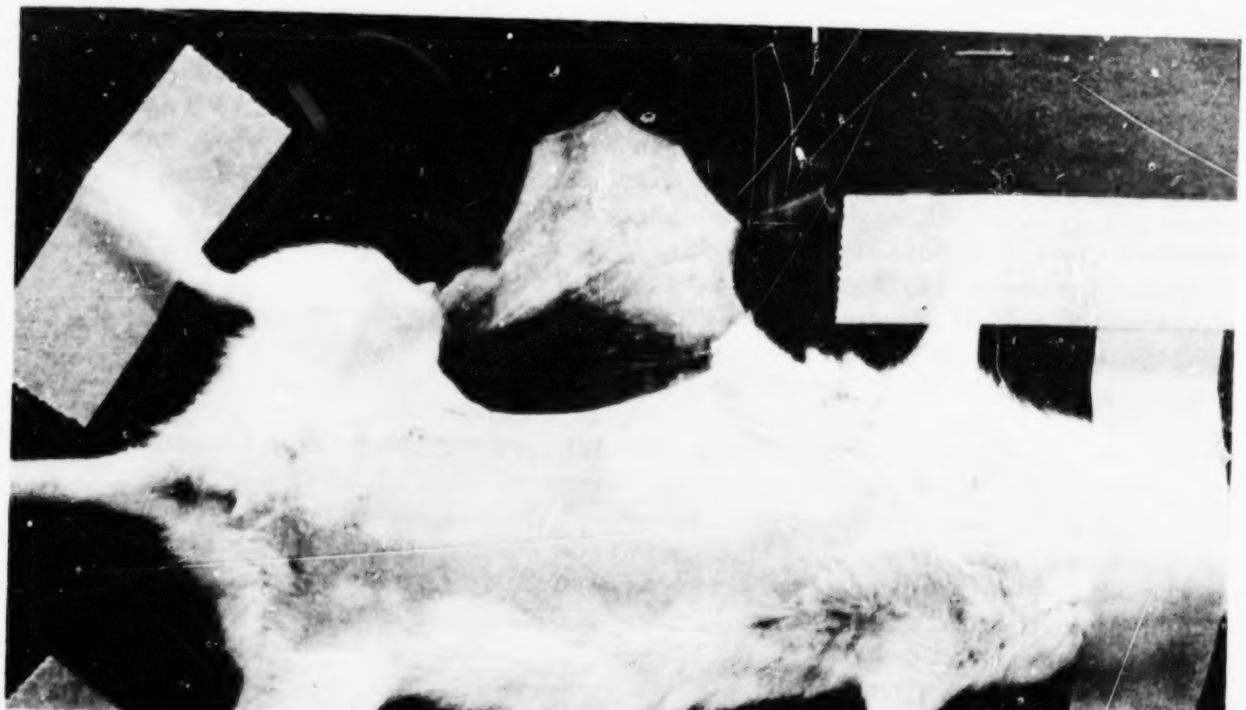

Figure 14. Externalized Flap of Mammary Tissue.

Results

The number of first mammary tumors appearing in the right inguinal, left inguinal, and the total number of mammary tumors are shown in Table 11. No statistical difference in the number of mammary tumors appearing in the right inguinal area could be demonstrated between the four treatment groups. This inability to show statistical differences between the treatment groups was concluded to be caused by the relatively small number of animals in the treatment groups.

Analysis of the number of mammary tumors appearing in the right inguinal as compared with the left inguinal area within each of the treatment groups showed statistical significance at the 95% confidence level in the whole-body minus flap group. However, the statistical significance was marginal, and in addition, a greater number of mammary tumors appeared in the area of mammary tissue that had received no radiation exposure.

The whole-body exposure to 100 rads increased the incidence of mammary neoplasia as was expected. There were 25 total mammary tumors in whole-body radiation groups as compared to 12 in the other groups.

After 18 months on the study 8 animals had died in the control group, 13 in the "flap only" group, 18 in the WB+F group, and 13 in the WB-F group. The major cause of death was respiratory infections.

Discussion

The carcinogenic effect of immune suppression caused by radiation and the direct effect of radiation damage to mammary tissue is regarded as a logical hypothesis. Previous investigation has shown that exposure to X irradiation in rats results in an incidence of mammary gland neoplasia greater than that found in controls.[5] It has been reported that a necessary prerequisite for radiation induced neoplasia of mammary tissue is direct radiation of breast tissue.[6] Certainly a parameter not determined in either of the previously mentioned experiments was the effect of the immune system on mammary gland neoplasia after partial or whole-body exposure.

Our data indicate that direct irradiation of the breast tissue is not a necessary prerequisite for neoplasia of the breast. It is difficult, however, due to the small number of neoplasms involved, to rule out that the four neoplasms that occurred in the right inguinal area in the whole-body minus flap group were not spontaneously occurring. It appears that factors other than pure radiation damage may be at play in radiation-induced mammary neoplasia, and that one of these factors may be the suppression of the immune mechanism.

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[5] Bond, V.P. et al. Rad. Res. 12:276-285 (1960).

[6] Bond, V.P. et al. Rad. Res. 13:318-328 (1960).

D. THE EFFECT OF DRUGS AND RADIATION ON THE INCIDENCE OF MAMMARY TUMORS IN OSBORNE-MENDEL RATS

Billy C. Ward, Lawrence R. Muroff, Joe R. Childress,
James N. Shively, Norman C. Telles

This study was initiated to determine the effect of various drugs--alone or in conjunction with radiation--on the natural incidence of mammary tumors in the Osborne-Mendel rat. Three drugs selected for the study were employed at high and low dose levels. These drugs were azathioprine (Imuran), dexamethasone, and ethionine, the ethyl analog of methionine. The dose levels, which were added to the diet, are indicated in the following chart:

Drug	Level	Dose (mg/kg body wt/day)
Imuran	High	9
Imuran	Low	2.25
Dexamethasone	High	0.03
Dexamethasone	Low	0.006
Ethionine	High	200
Ethionine	Low	100

A single whole-body exposure of 275 rads was given.

The animals were started on their respective diets on Nov. 15, 1968, and were irradiated either on December 2, or 3, 1968.

The preliminary data summarized in Table 12 were subjected to statistical analysis at approximately one year into the study (11/20/69). All fourteen treatments were examined. The results of this analysis indicated a significant chi-square value only in the comparison of irradiated to non-irradiated animals. These results indicate that radiation increased mammary tumor incidence, but neither choice of drug nor dose level of the drug had a significant effect. Statistical analysis has also shown that to date there is an absence of any synergistic effect of drug and radiation.

The study is still six months from completion and these results must be considered as preliminary.

TABLE 12. ANIMAL MORTALITY AND NUMBER OF TUMORS ONE YEAR AFTER EXPERIMENT BEGAN

DRUG	Dose level	Radiation	Number on study at start of exp.	Number dead	Of dead rats, number surviving 6 months	Of dead rats, number surviving 6 months with mammary tumor	Rats with one or more mammary tumors dead as of 11/20/69	Rats alive with one or more mammary tumors	Total animals with one or more mammary tumors
Ethionine	H	+	30	11	7	4	4	10	14
Ethionine	H	-	30	6	5	0	0	0	0
Ethionine	L	+	30	12	6	3	3	6	9
Ethionine	L	-	30	5	2	0	0	2	2
Imuran	H	+	40	13	6	2	2	9	11
Imuran	H	-	40	8	5	1	0	1	1
Imuran	L	+	40	6	6	6	6	10	16
Imuran	L	-	40	3	1	0	0	0	0
Dexamethasone	H	+	40	11	6	4	4	12	16
Dexamethasone	H	-	40	4	3	0	0	2	2
Dexamethasone	L	+	40	11	6	3	3	11	14
Dexamethasone	L	-	40	5	1	0	0	2	2
Control	-	+	30	7	3	2	2	5	7
Control	-	-	30	1	0	0	0	0	0

E. HOST CELL REACTIVATION OF UV-IRRADIATED VIRUS BY MAMMALIAN CELLS

C. D. Lytle

During the course of preliminary experiments designed to test if radiation can cause a normal lytic human virus to become oncogenic, it was discovered that Herpes simplex virus, a common human virus, is quite resistant to inactivation by ultraviolet light (UV). It is ten times less sensitive than the bacterial virus T2, which has approximately the same amount of DNA (the primary target for UV inactivation). Because UV is often used for sterilization purposes it was decided to investigate the factors contributing to the survival of UV-irradiated Herpes simplex virus.

Survival of this irradiated virus depends upon the cell system used for assaying infectivity (Fig. 15). This implies that the host cell plays a role in repair of radiation damage to the virus. Two essential features were found in the survival curves: The initial slope differs for different assay cells, and a second, more resistant component appears for some cell systems (e.g. CV-1 and HEp-2).

The different initial slopes very likely indicate different repair capacities of the different types of cells used. Using this interpretation it is concluded that green monkey kidney cells are more efficient at such repair than normal human skin or cancerous human larynx cells which are in turn more efficient than mouse fibroblasts. This is in agreement with the conclusion reached by Cleaver for human and mouse cells using repair replication abilities as repair criteria.

In the cases of CV-1 and HEp-2, the second components of the survival curves also follow the pattern that monkey cells are more efficient than human cells although the quantitative relation is different. If the second component lines are extrapolated to zero dose, the extrapolated number gives the fraction of the total plaque forming units which are in this resistant component. There is a correlation between the extrapolated number and the growth characteristics of the cells. Those cells which are still dividing at the time of infection (HEp-2 and some CV-1) have a higher fraction in the resistant component.

It is quite reasonable to think that the physiological state of the cell might control the activity or availability of repair enzymes. Indeed, Rauth had studied a repair process in mouse cells occurring during DNA synthesis. This repair can be inhibited by caffeine. Thus dividing cells (which are synthesizing DNA) might be expected (on the basis of Rauth's work) to have more repair than stationary phase cells. In our experiments caffeine has the effect of reducing the survival of the second component of virus survival without appreciably affecting the initial component (Fig. 16). Thus the resistant component of the virus survival curve is probably manifested by some viruses infecting dividing cells which have repair processes present during DNA synthesis.

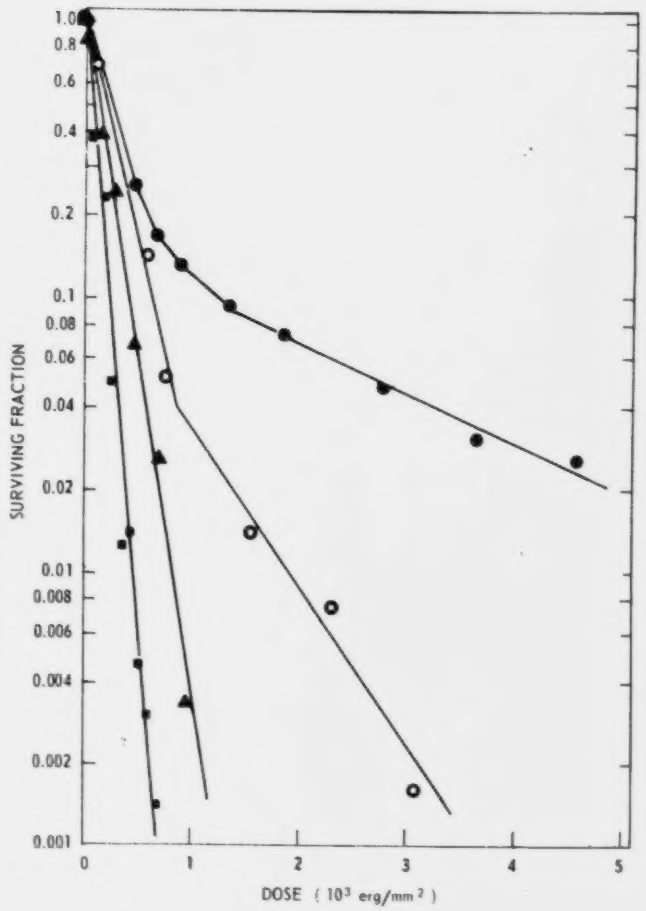

Figure 15. Survival Curve of Herpes Simplex Virus on Different Cells. ● - CV-1 (African green monkey kidney), ○ - HEp-2 (carcinoma from human larynx), ▲ - normal human skin fibroblasts, ■ - 3T3 (mouse fibroblasts).

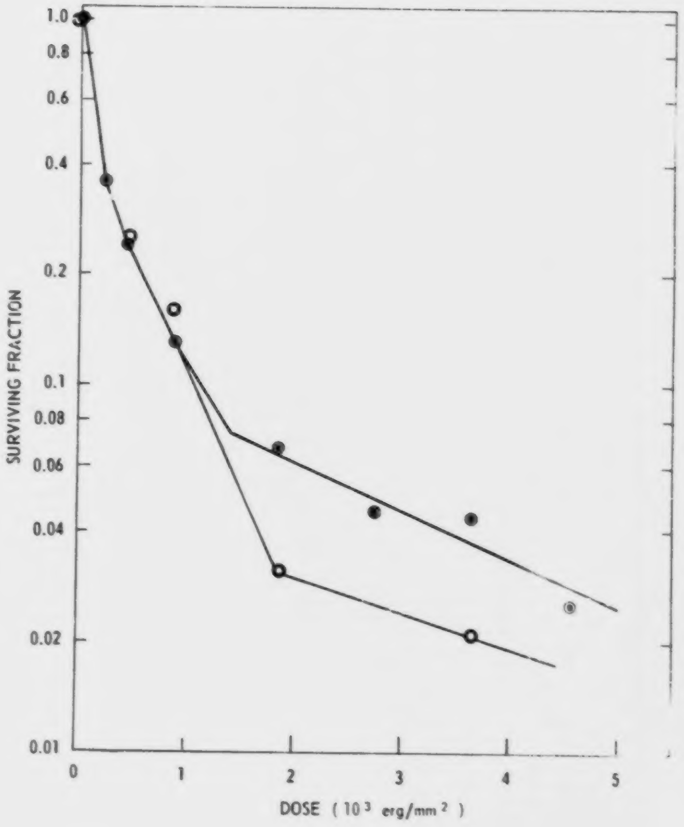

Figure 16. Survival Curves of Herpes Simplex Virus on CV-1 Cells in Presence (○) or Absence (●) of Caffeine (0.05%).

The time of appearance of the plaques differs greatly in the two components. The initial component plaques all appear at nearly the same time as unirradiated ones, but the resistant component plaques appear as much as 24 hours later. This is an indication that virus growth has been delayed for those viruses which appear more resistant to radiation. Our working hypothesis is that the irradiated virus (infecting those cells which have the repair during DNA synthesis) must wait for repair to occur before growth and infection can proceed.

In summary, host-cell reactivation has been found for a UV-irradiated human virus in mammalian cells. Part of this reactivation is sensitive to caffeine. Those Herpes viruses reactivated by the caffeine sensitive repair process cause plaques which appear later than normal.

The data presented here give quantitative relationships for repair efficiency among several mammalian cell types and perhaps make possible direct comparisons of radiation effects on normal and oncogenic viruses in model experimental systems.

F. THYROID AGE SENSITIVITY IN LONG-EVANS RATS

Joe R. Childress, Tom R. Horton, Billy C. Ward, Wah Lee,
Norman C. Telles

The methods, techniques and materials employed in the thyroid age sensitivity study have been described previously.[1] Briefly, this study allows rats of different age groups to experience a common post-exposure risk period over which tumors might develop. Particular attention has been given to preventing death of the animals in the age sensitivity study for more accurate statistical evaluation of thyroid tumor incidence.

The Long-Evans rats have been on the study approximately 21 months. Of the 960 females originally placed on the study, 295 have died. Figure 17 shows the number of dead irradiated and control animals within the 6, 3, and 1 month, and the 10 days of age treatment groups, respectively. It can be seen that more irradiated animals than control animals have died in the 3-month, 1-month, and 10-day groups.

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[1] Radiation Bio-Effects Summary Report, 1968, p. 45.

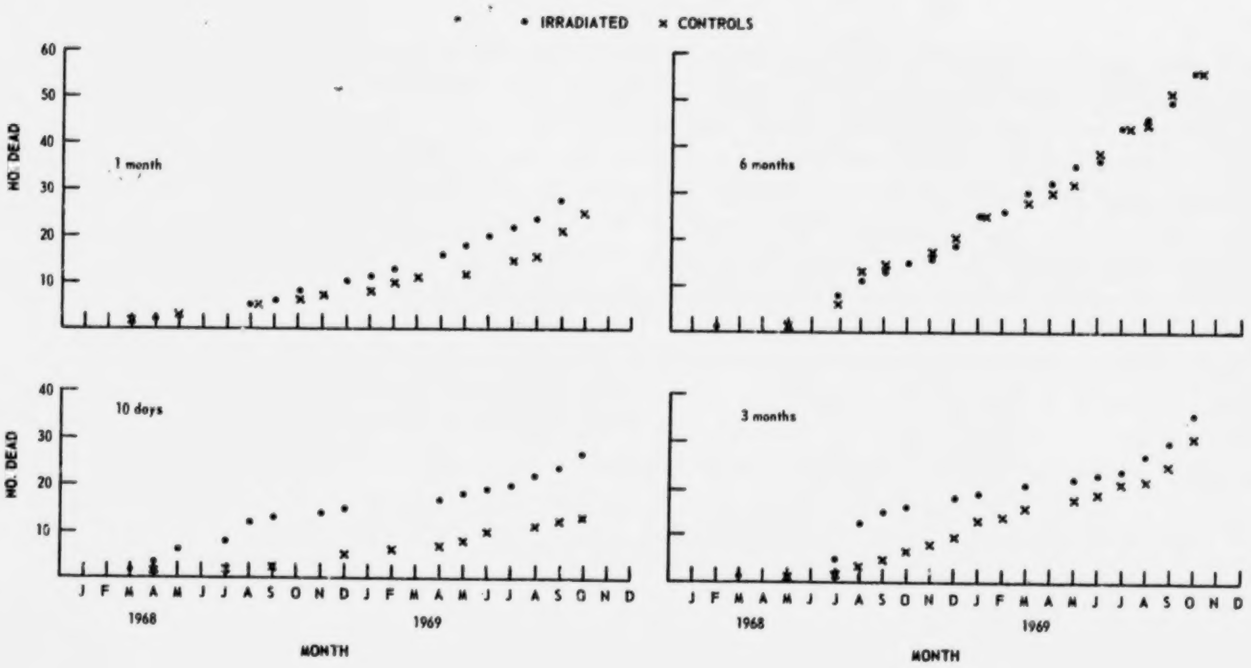

Figure 17. Numbers of Rats that Died During Course of Experiment.

On the systemic level, current data support the idea that younger tissue is more radio-sensitive than older tissue as indicated by the greater margin of difference between irradiated and control animals with decreasing age.

Although a small number of thyroid neoplasms have been observed, no comment can be made as yet concerning the relative age sensitivity of the rat thyroid gland. Interesting pathological findings include spontaneous lymphosarcomas (see below) and a large number of pituitary tumors. The pituitary tumors have appeared in experimental and control animals, generally in older rats.

G. SPONTANEOUS LYMPHOSARCOMA IN A COLONY OF LONG-EVANS RATS

Joe R. Childress, Billy C. Ward, James N. Shively

Although a variety of spontaneous tumors have been observed in rats, spontaneous lymphosarcomas are rare. Of 216 animals routinely necropsied from a colony of Long-Evans rats (see p. 71), spontaneous lymphosarcomas were found in 18 of the animals. Ten of the animals with lymphosarcomas had received X radiation to the thyroid (1000 rads, 250 kVcp and 15 mA). Eight of the animals were from control groups. Age of the rats at exposure, treatment groups, the number of animals dead, and the number of animals dead of lymphosarcomas within each treatment group are shown in Table 13. No statistical relationship between the occurrence of the lymphoma and X radiation of the thyroid gland could be demonstrated.

TABLE 13. COMPARISON OF OCCURRENCE OF SPONTANEOUS LYMPHOSARCOMAS TO AGE AT IRRADIATION

Age at Irradiation	Treatment[a]	Thyroid Dose (Rads)	No. Animals Dead	No. Lymphosarcomas
6 months	Experimentals	1000	50	4
	Controls	0	45	0
3 months	Experimentals	1000	28	2
	Controls	0	20	5
1 month	Experimentals	1000	29	3
	Controls	0	15	1
10 days	Experimentals	1000	19	1
	Controls	0	10	2

a. Each treatment group consisted of 120 rats.

The terminal clinical appearance of animals with lymphosarcoma was characterized by marked ascites, emaciation, and general malaise. Typical gross lesions were enlargement of the mesenteric and tracheobronchial lymph nodes. The spleen was normal. A generalized lymph node involvement was present in 2 of the 18 animals. One of these rats was an experimental animal; the other a control. These rats appeared to be leukemic, and their spleen was enlarged.

Histologic examination revealed a spectrum of changes. In typical cases having mesenteric and tracheobronchial node involvement, the malignant cells were immature lymphoblasts with large irregularly shaped vesicular nuclei with prominent nucleoli. The cytoplasm was basophilic. The malignant cells grew in a sheet-like arrangement. The cells were in various stages of mitosis. The point of origin of the malignant cells within the lymph nodes could not be determined due to the infiltrative nature of the cells. Hemorrhage and necrosis were commonly seen in the neoplastic nodes. The "starry sky" effect produced by the necrosis of individual cells was prominent.

In the leukemic animals, the blood vessels in almost every organ were choked with neoplastic lymphocytes. The meninges were heavily infiltrated. The neoplastic cells were moderately immature with round to oval nuclei with barely discernible nucleoli.

Electron microscopic studies to date have not revealed the presence of viral-like particles in the neoplasm. However, work is in progress to attempt to transmit these tumors by means of cell extracts of the lymphosarcoma to young Long-Evans rats.

H. RADIATION - AFLATOXIN CARCINOGENESIS

Frank Geiger, Jr.

Much work has been done in studying the effects of single carcinogens. However, in nature man may be exposed to multiple carcinogens. This study is being conducted to demonstrate whether a greater incidence of liver tumors occurs in rats receiving radiation plus aflatoxin as compared to either agent alone.

Aflatoxin is probably the most potent hepatoma-inducing agent known. There are several types of aflatoxin: B_1, B_2, G_1, G_2, M_1, and M_2 (B_1 being the most potent). Aflatoxin is predominately a metabolite of the fungus, Aspergillus flavus, but can also be produced by certain other fungi.

Aflatoxins B_1, B_2, G_1, and G_2 are carcinogenic to the rat, ferret, duck, and trout. More is known about the B_1 than the 3 other fractions. As a hepatotoxin, aflatoxin has been shown to affect cattle, chickens, dogs, ducks, guinea pigs, hamsters, pigs, mink, rats, trout, turkeys, and the rhesus monkey.

The fact that aflatoxin-producing strains of molds are found in many areas of the world and, furthermore, that climatic conditions favor the growth of molds in many areas of high liver carcinoma incidence lends support to the hypothesis that the aflatoxins are involved in the etiology of primary liver cancer. For example, aflatoxins have been detected at biologically significant levels in food samples collected from many parts of the world, particularly Africa and Asia.

Our studies utilize the weanling, female Osborne-Mendel rat. Initial studies are being performed to determine the level of aflatoxin B_1 and B_2 which can be tolerated by stomach intubation without causing death by acute toxicity but will produce a low incidence (about 10%) of liver tumors at the end of about 1 1/2 to 2 years. In each study rats will be stomach intubated with water, solvent (propylene glycol), aflatoxin B_1 and aflatoxin B_1 plus B_2. Each of these groups will either have received 300 rads of whole-body X radiation or will not have received any irradiation 3 weeks prior to treatment. Each group will contain 10 animals so that statistical analysis can be made between any of these groups. After 21 days of intubation the rats will be sacrificed and studied grossly and microscopically. Acute effects can then be compared with chronic changes.

A larger study will follow with 600 rats. These rats will not be sacrificed unless there is an obvious tumor and the animal is moribund. Rats dying before one year will not be counted since hepatoma induction would not have had its average time for appearance.

Rats will also be checked for mammary tumors to complement the Radiation - Ethionine Co-Carcinogenesis study performed previously (Radiation Bio-Effects Summary Report, Jan. - Dec. 1967, p. 54).

I. EFFECTS OF AGE ON RADIATION MORTALITY IN THE CHINESE HAMSTER

Billy C. Ward, Joe R. Childress, Gordon L. Jessup

Chinese hamsters (*Cricetulus griseus*) from the Bureau of Radiological Health colony were exposed to whole-body radiation at approximately 12 hours, and 3, 7, 15, 71 and 100 days of age. Each age group was exposed to 250 kVcp filtered X rays at a constant dose rate of 16.6 ± 0.1 rads per minute at various doses ranging from 500 to 1050 rads. The 1,467 hamsters exposed during this experiment were confined in a partitioned, rotating lucite wheel. The hamsters were maintained in environmentally controlled rooms and examined daily for 30 days following exposure or until death.

Median time to death of decedents, mortality curves and $LD_{50/30}$ values were determined for each age group. $LD_{50/30}$ values for the various groups were 635 rads (12 hours), 766 rads (3 days), 785 rads (7 days), 849 rads (15 days), 881 rads (30 days), 918 rads (71 days), and 983 rads (100 days).

The effect of age on the $LD_{50/30}$ is shown in Figure 18, where the $LD_{50/30}$ values are plotted in relation to age. The broken line connects the $LD_{50/30}$ values, and the vertical lines represent the 95% confidence limits at the various ages. The sharpest rise in radioresistance occurred during the first 3 days after birth. After the first 3 days of age, a slow but steady increase in radio-resistance occurred until the hamsters reached 100 days of age which was the oldest age group tested in this experiment. Work is now in progress to determine the effect of age on radiation mortality in hamsters more than 100 days old.

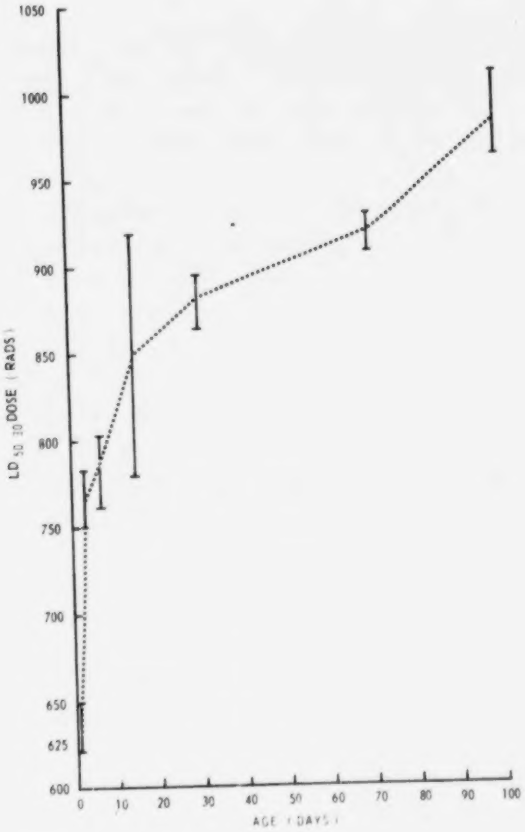

Figure 18. Effect of Age on $LD_{50/30}$ in Chinese Hamsters.

J. CATARACTS INDUCED BY 30 KVP AND 250 KVP X RAYS

James Shively[1], James Rolofson, Clay Cisar with the collaboration of Seth Koch[1]

Introduction

Induction of lenticular opacities has been recognized for many decades as a potential hazard of exposure to ionizing radiation. A vast amount of literature about ionizing radiation induced cataractogenesis exists; however, almost all of these studies have used laboratory rodents exposed to high-energy neutrons or gamma rays.

With the advent of interest in the potential hazard from electronic devices emitting lower-energy X rays, an attempt to evaluate induction of cataractogenesis by these sources became appropriate. Swine were selected as experimental subjects in an attempt to simulate the human eye more closely since the relative dimensions, circulation, and structure of swine eyes are more like those of man than are those of other experimental animals (except subhuman primates).

Materials and Methods

Sixty weaned, mixed breed 10- to 12-week-old swine were purchased and were quarantined for 3 weeks to allow time for incubation of any diseases to which the swine might have been exposed before their eyes were irradiated. The swine were kept in a barn having concrete floors[2]. A commercial hog ration was fed to maintain normal growth rates. Water was available _ad libitum_.

Surgical anesthesia was induced by the intravenous injection of pentobarbital sodium to maintain immobility of the swine during irradiation of the ocular area. The swine were placed in lateral recumbency for irradiation. The beam was collimated and a lead shield was positioned to prevent exposure of tissue other than the globe and periorbital structures (Fig. 19). Each eye of the swine was exposed to an absorbed dose at the midpoint of the lens of 100, 600, or 1,000 rads of 30 kVp or 250 kVp X rays.

The 30 kVp exposures were done with a low energy (0-75 kVp) X-ray machine operating at 30 kV constant potential, 90 mA, 25 cm T.S.D. with 3.09 mm aluminum filtration added to a collimated beam. The absorbed dose rate was approximately 38 rads/minute. For higher energy

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[1] Veterinary Ophthalmologist, Eye Research Foundation, Bethesda, Maryland, and the Animal Hospital of Penn Daw, Alexandria, Virginia.

[2] The swine were kept at the National Institutes of Health Animal Center under contract with the Laboratory Aids Branch, National Institutes of Health.

X irradiation, a 250 kV constant potential X-ray machine was used operating at maximum energy, 7.0 mA, 20 cm T.S.D. with a Thoraeus filter and 0.42 mm lead added to the collimated beam. The dose rate at the midpoint of the eye lens was approximately 56 rads/minute.

Eyes were collected from some swine 3 or 10 days after irradiation. Surgical anesthesia was induced with pentobarbital sodium intravenously and the eyes were quickly enucleated. The swine were then killed. For electron microscopy one eye was hemisected transversely posterior to the lens and the rostral calotte was immediately placed in 6.25% glutaraldehyde in phosphate buffer (pH 7.3) with 2% acrolein added. Fixation was continued for 90 minutes after which the calotte was transferred to phosphate buffer, pH 7.3. Blocks about 1 x 1 x 3 mm were cut from the anterior lens epithelium, equatorial lens, and the posterior position of the lens. Post-fixation in 2% OsO_4 in veronal buffer was then performed. The blocks were dehydrated in a series of ascending concentrations of alcohol and embedded in epon.

For light microscopy one whole eye was fixed for 72 hours in the solution used for fixation for electron microscopy. The globe was hemisected to produce 2 callotes, nasal and temporal. These were dehydrated and embedded in paraffin or celloidin. Sections were cut and stained for examination with the light microscope.

Periorbital tissue and ocular adnexa were collected for light microscopy and a complete necropsy was done on each swine killed.

Half the swine are being kept for longer term study of development of lenticular opacities. For about 3 months after irradiation the eyes were examined with an ophthalmoscope. Then the collaboration of a veterinary ophthalmologist (SK) became available and periodic examinations by biomicroscopy and indirect ophthalmoscopy were done (Fig. 20).

Results

Periorbital erythema became apparent within a day after the higher doses of radiation. In some swine mild conjunctivitis developed within a few days and transient clouding of the cornea occurred. Clouding of the cornea cleared about 14 days after irradiation. The conjunctivitis has persisted in some swine.

Clinical examinations have not revealed significant lenticular alterations to date. The results of morphologic studies are inadequate in amount to allow reporting at this time.

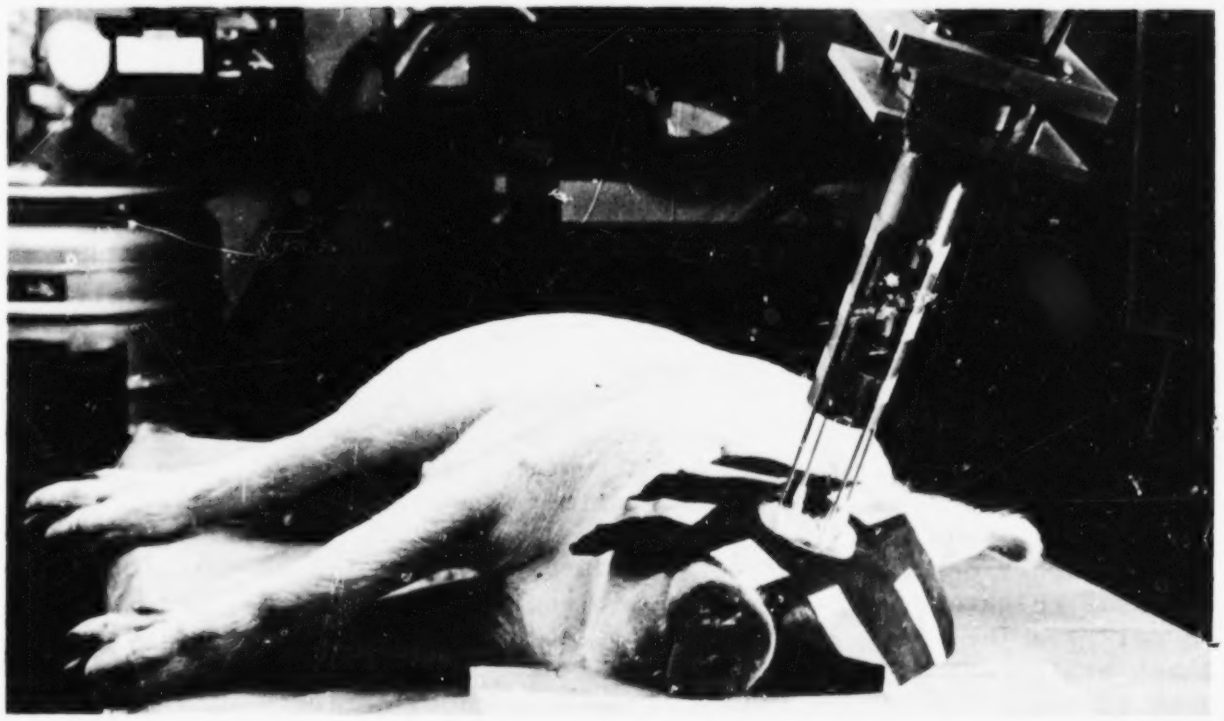

Figure 19. The Eyes of Swine Were Exposed to 250 or 30 kVp X Rays To Study Radiation-Induced Cataractogenesis.

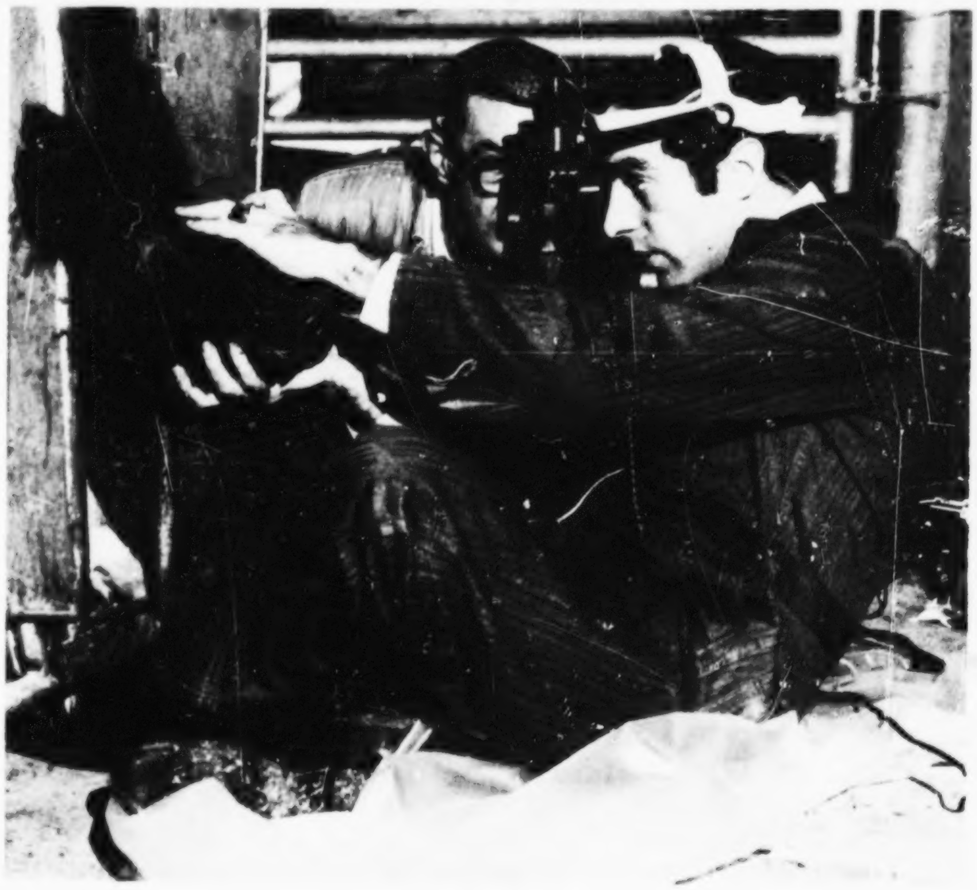

Figure 20. Swine Were Examined by Biomicroscopy and Indirect Ophthalmoscopy in a Study of the Effects of Ocular Irradiation.

K. X IRRADIATION OF BALB/3T3: SARCOMA FORMING ABILITY AND VIRUS INDUCTION

Edward J. Pollock, Stuart A. Aaronson[1], George J. Todaro[1]

The occurrence of a variety of tumors following large doses of radiation has been well documented for man and animals. Leukemia induced by irradiation in certain strains of mice has been shown to have a viral etiology.[2] In tissue culture systems, it has been demonstrated that radiation of cells increases their susceptibility to transformation by several oncogenic DNA viruses. At the doses used in these papers radiation alone produced no detectable alterations in cellular morphology. The only report of transformation by X irradiation in the presumed absence of virus is that of Borek and Sachs using primary hamster embryo cells.[3]

This report describes the effects of high doses of X irradiation on the tissue culture properties and tumorigenicity of Balb/3T3 (mouse embryo cells). The survival of 3T3 cells at high radiation doses is low but can be increased by irradiating confluent rather than dividing cells and by allowing time to permit repair before subculturing. In this way the effect of a high dose (1,500 rads), comparable to doses that have been shown to induce solid tumors in animals and man, can be examined. Irradiation of cells in tissue cultures eliminates many of the variables encountered in studying radiation carcinogenesis in the whole animal.

Cultures were irradiated with a 250 kVp X-ray therapy machine at a dose rate of 150 rads/min. Confluent monolayers were irradiated with 1500 rads and 24 hours later were subcultured at 10^3 cells/50 mm petri dish. Unirradiated control Balb/3T3 monolayers were transferred to petri dishes at the same cell density.

The colony forming ability (CFA) of control Balb/3T3 is approximately 30 percent; 1,500 rads reduced this survival to 0.5 percent. Most of the surviving colonies from irradiated cultures were indistinguishable from typical Balb/3T3. They showed the characteristic epitheloid morphology and high degree of contact inhibition of cell division. A small fraction (0.8 percent in this experiment) of the surviving colonies could be recognized as having transformed morphology. One such clone, R_4, as well as a randomly selected subclone of Balb/3T3, subclone C_3, and a radiation treated subclone with normal morphology, R_1, were used in the in vitro and in vivo studies. Cloned lines were developed from each of these colonies and were grown up through a period of 20-30 cell generations. Tumorigenicity was then tested by inoculating 10^6 and 10^7 cells subcutaneously into the interscapular area of weanling Balb/c mice (irradiated with 350 rads, whole body).

Control Balb/3T3 and subclone C_3 made no tumors. Similarly no tumors appeared in nine animals injected with subclone R_1. Subclone R_4, which had a higher saturation density in tissue culture (Table 14), produced progressively growing tumors at the inoculation site with 10^7 cells in 17 of 20 animals. The tumors that developed all had the histological appearance of highly undifferentiated fibrosarcomas.

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[1] National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[2] Kaplan, H.S., Cancer Research, 27, 1325 (1967)

[3] Borek, C. and Sachs, L., Nature, 210, 276 (1966)

TABLE 14. TUMOR INDUCTION BY IRRADIATED AND UNIRRADIATED BALB/3T3 CELLS

	Saturation Density[a] ($x 10^{-6}$)	Number of Animals with Tumors/ Number Inoculated[b]	
		10^6	10^7
Balb-3T3-Cl 31	1.0	0/35	0/20
Subclone R_1	1.2	--	0/9
Subclone R_4	3.1	4/8	17/20
Subclone C_3	1.1	0/4	0/13

a. Final cell number per 20 cm^2 petri dish.
b. Observation period - 6 months.

We tested the cell-inoculated Balb/c animals for the presence of virus particles and infectious virus. Electron microscopy revealed typical budding C-type particles in the R_4 induced tumors and in cell lines derived from the tumors. Sucrose density gradients of supernatants from cultures of tumors incubated with 10 μCi/ml of uridine - H^3 showed a sharp peak at 1.16 g/cm^3, the density of viruses of the murine leukemia - sarcoma complex. Recent experiments have shown that virus can be isolated from an R_4 - induced tumor by passaging cell-free extracts on primary mouse embryo cultures. Cell homogenates of spleens from subclone R_4 and subclone C_3 inoculated animals were tested for complement fixation antigen activity against murine leukemia (MuLV) group reactive antiserum. All R_4 inoculated animals were positive in this test; the C_3 inoculated animals and the uninoculated controls were all negative. The R_4 cells, however, have not shown evidence of infectious virus production in tissue culture; Balb/3T3 and subclone C_3 have also been negative for virus.

In this report it is shown that a single exposure to a physical agent, X radiation, is capable of producing an alteration of tissue culture properties. Borek and Sachs[3] were able to recognize transformed colonies of mass cultures of hamster embryo cells irradiated with 300 rads and found no such colonies in control plates. The low radiation dose they used produced little cell killing, and they concluded that the transformants were directly induced by irradiation.

Concurrent studies with long-term cultures of Balb/c embryo and with random bred Swiss embryo cells have shown that certain cell lines which have lost contact inhibition and have become tumorigenic begin, apparently spontaneously, to release mouse leukemia virus. This finding, along with the detection of virus in aged Balb/c mice has suggested the hypothesis that the mouse leukemia-sarcoma genome might be present, in a repressed state, in many and perhaps in all mouse embryo cells. If this is the case, high radiation doses may favor the expression or activation of a repressed virus. However, in these experiments, proof that the virus in the tumors came from the inoculated cells rather than from the host will require further investigation.

NEUROPHYSIOLOGIC STUDIES SECTION
Dr. Norman C. Telles, Acting Chief

Among the more interesting and potentially most important effects of low-energy-density microwave radiation are those which have been ascribed to the interaction of microwaves with the central nervous system. A program is currently being developed within the Neurophysiologic Studies Section in which these interactions will be investigated; the investigations will involve the use of physiologic as well as psychometric testing and evaluation techniques. The following report summarizes the most recent activities of the Neurophysiologic Studies Section. The future direction of this work is summarized at the end of the report.

CEREBRAL EFFECTS OF RADIO FREQUENCY ENERGY
Maitland T. Baldwin, William P. Edwards

Introduction

In June of 1969, work relating to cerebral power levels and head position during exposure to radio frequency energies was reported at the International Congress of Industrial Neurology held in Prague. In essence, this report provided two sets of observations: (1) Power levels ranging from 10-20 mW were recorded from the right or left parietal lobe during controlled exposures of the living macaque rhesus head in a resonant cavity with an average power of less than 30 W delivered in the field; (2) the cerebral power levels varied in accordance with the head position in the field. Thus, when the head faced the antenna and was elevated 30°, the power in the brain was highest.

These findings suggested that orientations of persons exposed in radio frequency fields might influence the power to which the brain was exposed and thus the nature of effects on cerebral activity during and after exposure.

Obviously, these findings were dependent on the characteristics of the cavity, with particular relationship to operating frequency, resonance, load characteristics, and load measurement techniques. In addition, geometric relationships of head holder to the field were considered significant, as were ambient humidity and temperature.

These factors required analysis to confirm and extend the findings reported in June of 1969 and prepare them as a basis for further experimentation.

Cavity Characteristics

Construction

The right circular cylinder cavity resonator used in present and forthcoming experiments has a radius of 47.92 cm and a height of 45.2 cm. The walls are regular window aluminium screen, 16 mesh per inch, and the top and bottom plates are 1/8- and 1/4-inch-thick aluminum sheets. Three one-inch diameter aluminum rods extend from the bottom plate to support the cavity 41 inches from the floor (Figure 21). A comfortable monkey chair is attached to the three rods. The center of the bottom plate has a 9-cm diameter hole through which the monkey's head protrudes. By the proper selection of neck collars, an RF tight metallic fit can be made to minimize the energy loss during exposure.

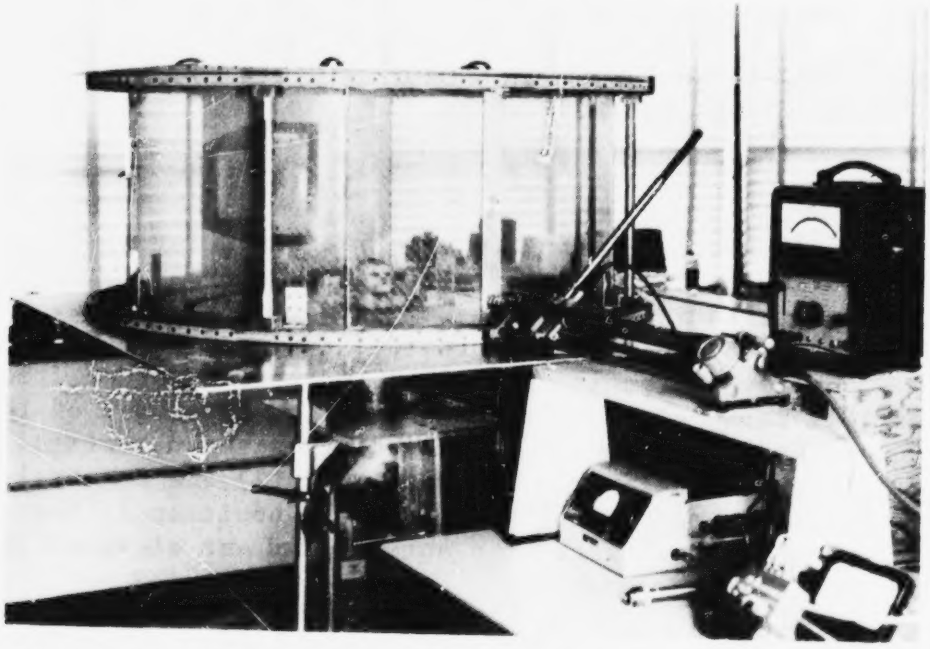


Figure 21. Resonant Cavity Used to Irradiate Monkeys.

Modification

The cavity was modified to allow the bottom plate assembly and monkey to revolve 360 degrees so that any angle of the monkey's head could be directed toward the incident electric field vector without discontinuing the exposure sequence. This was accomplished by cutting the bottom plate to a radius of 16 inches and placing it between two other aluminum sheets so that centering and shielding were maintained. A wooden platform was made to support the entire cavity assembly.

Operating Frequency

The dimensions previously stated are related to the resonant frequency by

$$\lambda = 2\left[\left(\frac{2X_{LM}}{\pi d}\right)^2 + \left(\frac{1}{L_c}\right)^2\right]^{-\frac{1}{2}}$$

where: λ is wavelength in meters
X_{LM} is a Bessel function = 1.841 (TE_{111} mode)
L_c is the cavity length
d is the cavity diameter.

Solving for λ:

$$\lambda = 2\left[\left(\frac{2 \times 1.841}{3.14 \times .9588}\right)^2 + \left(\frac{1}{.452}\right)^2\right]^{-\frac{1}{2}}$$

$$\lambda = .7913 \text{ meters.}$$

Since λ is related to frequency in MHz by $\lambda = \frac{300}{\text{freq}}$,

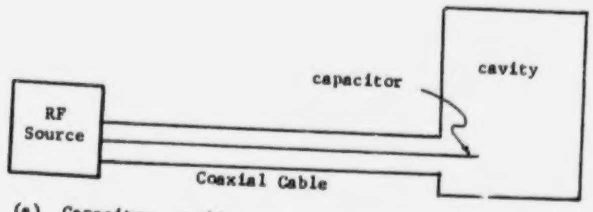
$$\text{frequency} = \frac{300}{.7913} = 379.1229 \text{ MHz.}$$

By varying the dimensions of the cavity, resonance can be made to occur from approximately 370 to 390 MHz.

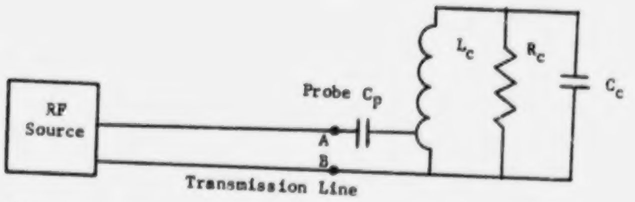
Cavity as a Load

The cavity is coupled to the RF source by a length of RG-8/u coaxial cable which terminates in a one-quarter wavelength probe that extends through the cavity wall 6 centimeters above the bottom plate. This position of excitation yields the highest electric field vector in the direction of the E field (see Fig. 22c) approximately 6 centimeters high. (The monkey's brain stem lies in approximately this plane.) Figure 22a shows the basic circuit designed to deliver power to the cavity. Figure 22b represents the equivalent electrical circuit and Figure 22c shows the field configuration inside the cavity. From the equivalent circuit it can be determined that the impedance reflected to the transmission line is a function of the effective values of the cavity loss (R_{eff}) and the effective values of the resultant reactive components L and C. This impedance is coupled to the transmission line through the capacitor probe (C_p) in the complex form of $R \pm jX_t$, where X_t is the effective value of all reactive components at the operating frequency. If the line is opened at points A and B (Fig. 22b), and a slotted line inserted, the reflected impedance can be measured to an accuracy of within ten percent of the true value. There were noticeable changes measured here due to changes in the relative humidity, the placement of the monkey's head and head holder within the cavity. Changes in the impedance were also observed due to orientational changes in the head and head holder. The head holder is made of four pieces of acrylic plastic, two pieces 4 x 1 x 1/2 inches and the other two pieces 2 x 2 x 1/2 inches held together by 8 nylon 1/4 x 20 screws 1 inch long. If the frequency is held constant, the parameters of the cavity are constant and changes in the measured impedance vary with respect to changes in the above conditions.

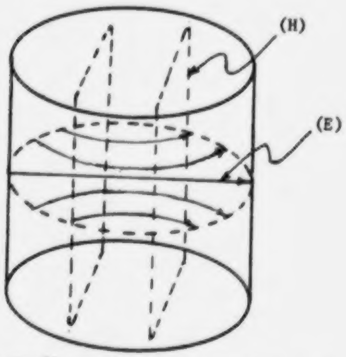
Phasor diagrams of the impedance changes as a function of changes in the relative humidity and the monkey's head and head holder orientations are shown in subsequent sections.



(a) Capacitor coupling cavity resonator



(b) Equivalent circuit of the capacitor-coupled cavity



(c) Cavity field configuration for the TE_{111} mode

Figure 22. Diagrams of Irradiation Cavity. (a)Basic circuit for delivering power to the cavity, (b) equivalent electrical circuit, (c)field configuration inside the cavity.

Load Measurements Techniques

When a transmission line is terminated with a load Z_L, and if Z_L equals the characteristic impedance of the transmission line Z_o, the voltage standing wave ratio (VSWR) is equal to one and the impedance at every point on the line is equal to Z_o. If Z_L differs from Z_o, then the VSWR is greater than one and the impedance is changing everywhere on the line. The impedance reaches maximums of (Z_o) (VSWR) resistive and minimum values of Z_o/VSWR resistive. At any place between these two points the impedance is either inductive or capacitive in nature. The relationship that exists between the impedance at any point on the line Z_p and the impedance of the load connected to the line Z_L and the characteristic impedance of the line Z_o is:

$$Z_p = Z_o\left[\frac{Z_L + jZ_o \tan\theta}{Z_o + jZ_L \tan\theta}\right]$$

Figure 23 shows the impedance along an unmatched line for a length of one and one-half waves. Notice that Z_p maximum is located somewhere between the arc AB, whereas Z_p minimum lies between the arc CD. It is obvious that Z_p minimum can be more accurately located than Z_p maximum.

Rearranging the preceding equation we obtain:

$$Z_pZ_o + jZ_pZ_L\tan\theta = Z_oZ_L + jZ_o^2 \tan\theta.$$

Factoring out Z_L and Z_o and multiplying by -1, we obtain:

$$Z_L(Z_o - jZ_p \tan\theta) = Z_o(Z_p - jZ_o \tan\theta).$$

Dividing:

$$Z_L = Z_o\left[\frac{Z_p - jZ_o \tan\theta}{Z_o - jZ_p \tan\theta}\right]$$

Substituting Z_o/VSWR for Z_p:

$$Z_L = Z_o\left[\frac{\frac{Z_o}{VSWR} - jZ_o \tan\theta}{Z_o - j\frac{Z_o}{VSWR}\tan\theta}\right]$$

Multiplying numerator and denominator by $VSWR/Z_o$ gives:

$$Z_L = Z_o\left[\frac{1 - j\,VSWR\,\tan\theta}{VSWR - j\,\tan\theta}\right]$$

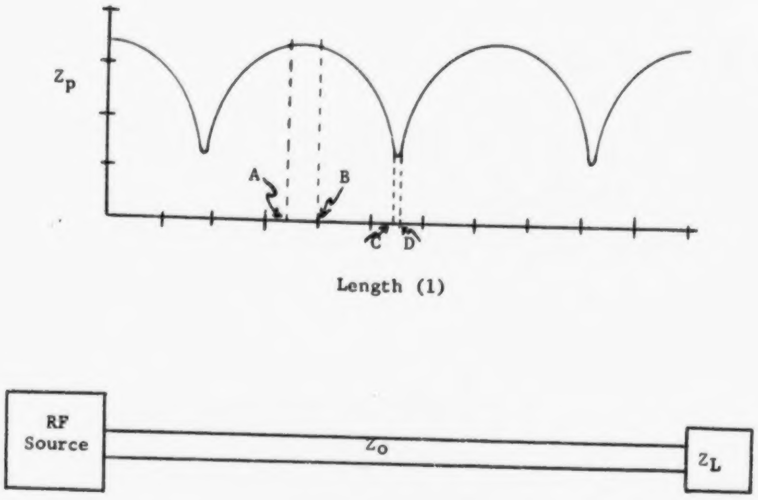
This equation was programmed into a calculator and the solution was given out in both rectangular and polar coordinate forms. The two members of the equation can therefore be determined by measurements readily available on the slotted line.

Determining Tan θ

$$\theta = \left[\frac{L\sqrt{K}}{\lambda}\right] 360^\circ$$

where: L is the length in centimeters between the load and the first impedance minimum point on the transmission line (Fig. 23), K is the dielectric constant.

The value of θ can be determined to an accuracy of the nearest ten minutes of arc.



Impedance along an unmatched line ($Z_L = Z_o$)

Figure 23. Impedance along an Unmatched Line for a Length of One and One-Half Waves.

VSWR Determination

In order to determine the VSWR values, the RF voltage at maximum points and at minimum points must be measured. The following circuit (Fig. 24) was constructed and connected to the adjustable probe on the slotted line (General Radio Type 874-LBA). The probe was adjusted to a depth of about 3 mm into the slotted line (approximately 30 per-cent of the distance between the two conductors), a short circuit was placed at the load end of the line, and RF power was increased until 1 millivolt, 10 millivolts, and 100 millivolts on the DC vacuum tube voltmeter (Hewlett Packard Model 412A) was measured. The probe was moved in 10-degree steps for a distance of 90 degrees of a cycle for each of the voltage magnitudes mentioned. A graph was plotted showing the voltage values recorded as the ordinate and the 90 degrees in 10-degree steps as the abscissa. To show the linear and square law region a pure sine function is also shown (Fig. 25). The curve shows clearly both the linear (-•-•-) and square law region of the crystal output characteristic. The true sine squared function lies nearly midway between 1 and 10 millivolt curves (not shown). Both regions of the crystal output voltage were used in determining the voltages measured for the VSWR. For low values (VSWR less than 10) the 100 mV linear region was used.

$$\text{VSWR} = \frac{100 \text{ mV}}{E_{min}}$$

where: E_{min} is the measured value corrected by the graph.

When the VSWR exceeded 10, the square law region and the approximating equation

$$\text{VSWR} \simeq \frac{\lambda}{\pi\Delta} \text{ was used}$$

where: Δ is the distance in centimeters between successive double power points.

These points were found to be 1.815 times the value of E_{min} for power levels from 2 to 4 milliwatts using the circuit shown in Figure 24. Justification for the alternate methods lies in the fact that the probe coefficient of coupling remains more nearly constant in the relatively uniform field strength areas in the slotted line.

Reflected Impedance Measurements

If the cavity were operated such that the impedance presented to the line was R ± j0, the distance between the load and the first

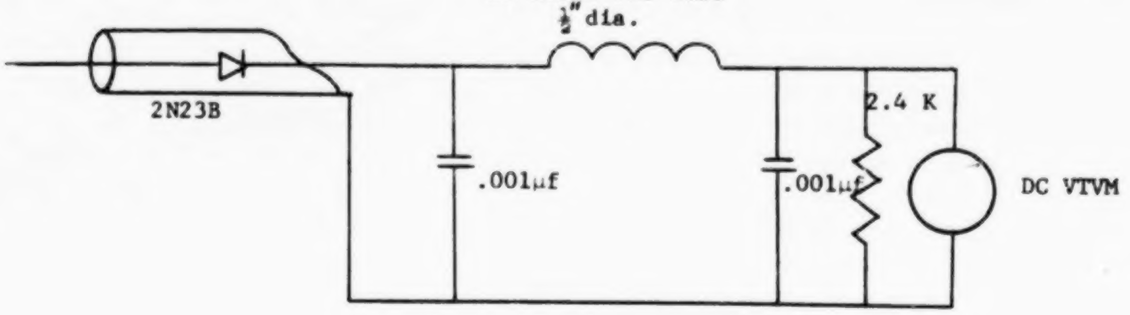

10 Turns #18 Wire
½" dia.
2N23B
.001µf
.001µf
2.4 K
DC VTVM

Figure 24. VSWR Detector and Indicator.

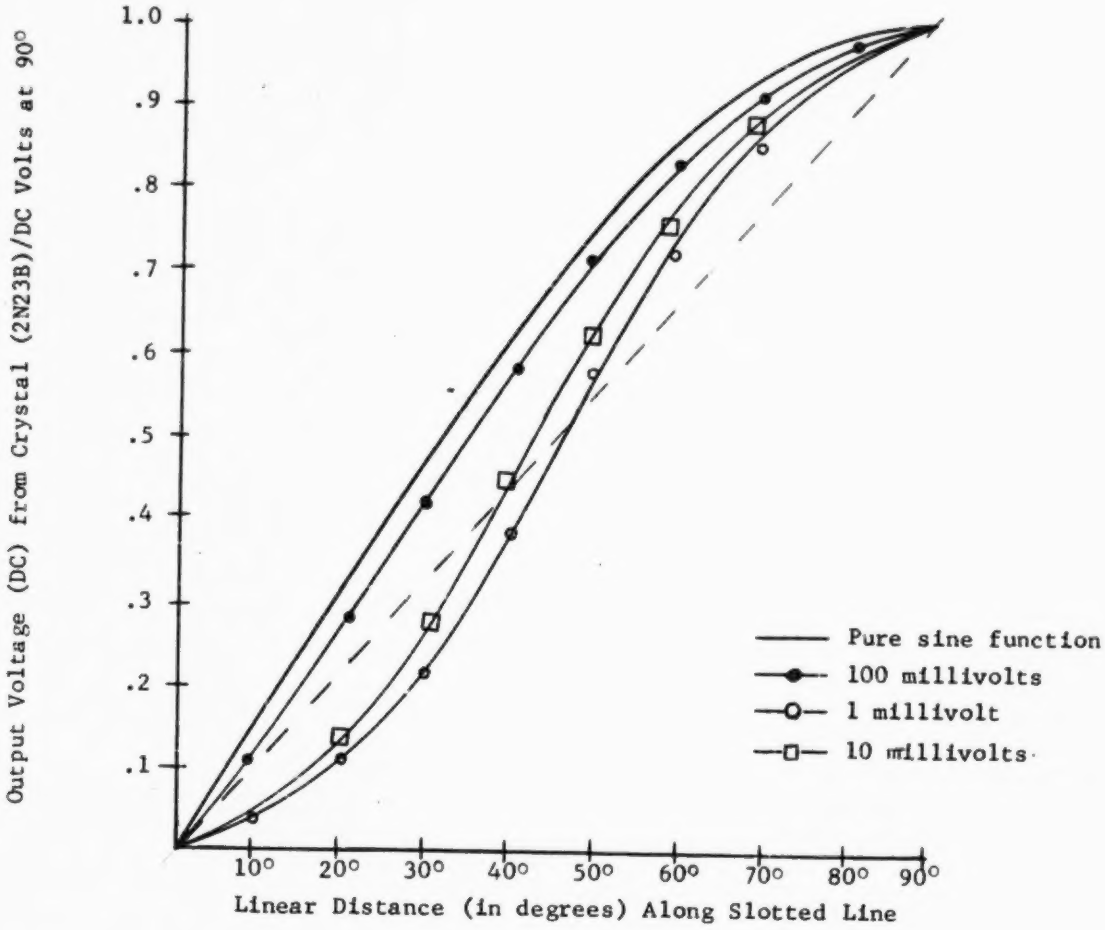


Figure 25. Crystal Characteristics Vs. Input Voltage Levels.

impedance minimum on the line in electrical degrees could result in first or second quandrant angles depending on whether the desired variables added an inductive or capacitive component. To work with all first quandrant angles, the empty cavity(at 24° C, relative humidity 59.9 percent) operating at 385 MHz was tuned to reflect an impedance of 5.673-j69.223 ohms. This is used as the reference for the succeeding measurements.

Loading Impedance of the Loaded Cavity

The empty cavity was loaded by first placing the monkey's head holder in its normal center position with its two 4-inch long mouth bits support bars (plastic) parallel to the bottom plate and directed toward the E field. The reflected impedance was then measured. The head holder was elevated 30 degrees with respect to the bottom plate and again measurements were made. The monkey's head was placed in the head holder and measurements were taken in the two positions described above. The following series of measurements was made:

Experimental Condition Variables	Reflected Impedance
Empty cavity	5.673-j69.223
Cavity, head holder at 0°	12.491-j62.967
Cavity, head holder at 30°	11.097-j63.042
Cavity, monkey, head holder at 0°	21.240-j28.750
Cavity, monkey, head holder at 30°	21.320-j32.453

By subtracting the phasor components the reflected impedance caused by each of the variables is:

Experimental Condition Variables	Reflected Impedance
Head holder at 0°	6.818+j6.246
Head holder at 30°	5.424+j6.181
Monkey's head at 0°	8.749+j34.217
Monkey's head at 30°	10.223+j30.589

Figure 26 shows a plot of the above reactive components as a function of real resistance.

Cavity Impedance Measurements vs. Humidity

The cavity was placed in a sealed chamber so that the relative humidity could be changed from 10 to 95 percent, while temperature changes were held at 29 ± 1.0° C. The cavity was tuned to reflect an impedance of 19 ± j0 ohms while having a relative humidity of 50 percent at 28.2 degrees. The humidity was then dropped to 30 percent and increased in small steps to 92 percent. During each step measurements were taken. Figure 27 shows a plot of the

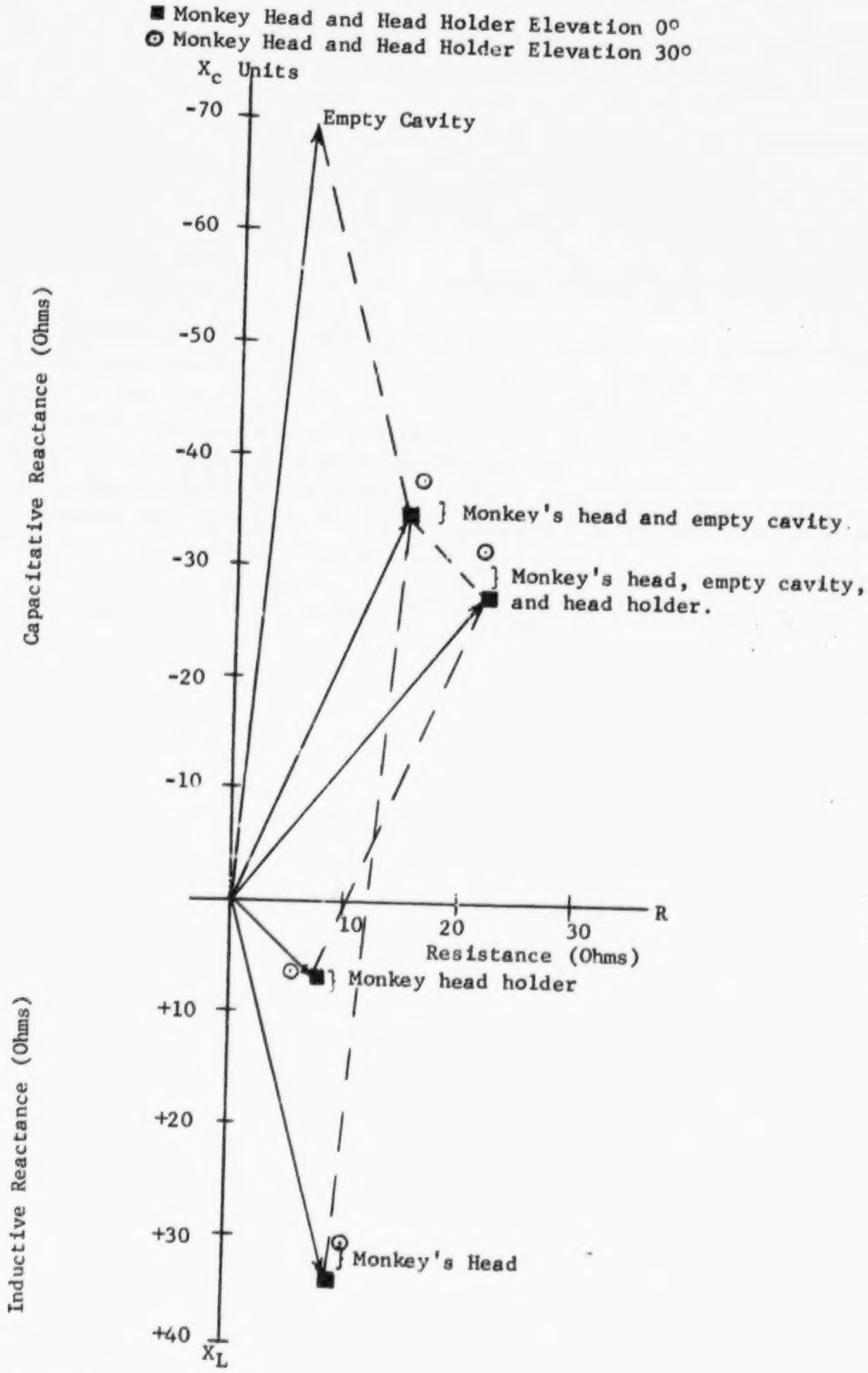


Figure 26. Reflected Cavity Impedance Phasor Diagram.

absolute magnitude of the impedance and the phase angle as a function of relative humidity. Note that there occurs a shift in the phase angle of the impedance without any apparent significant change in the absolute magnitude of the impedance. This shift occurs at about 60 percent relative humidity. Note also that an apparent decrease in the absolute magnitude of the impedance occurs without any obvious phase angle shift at 70 percent relative humidity.

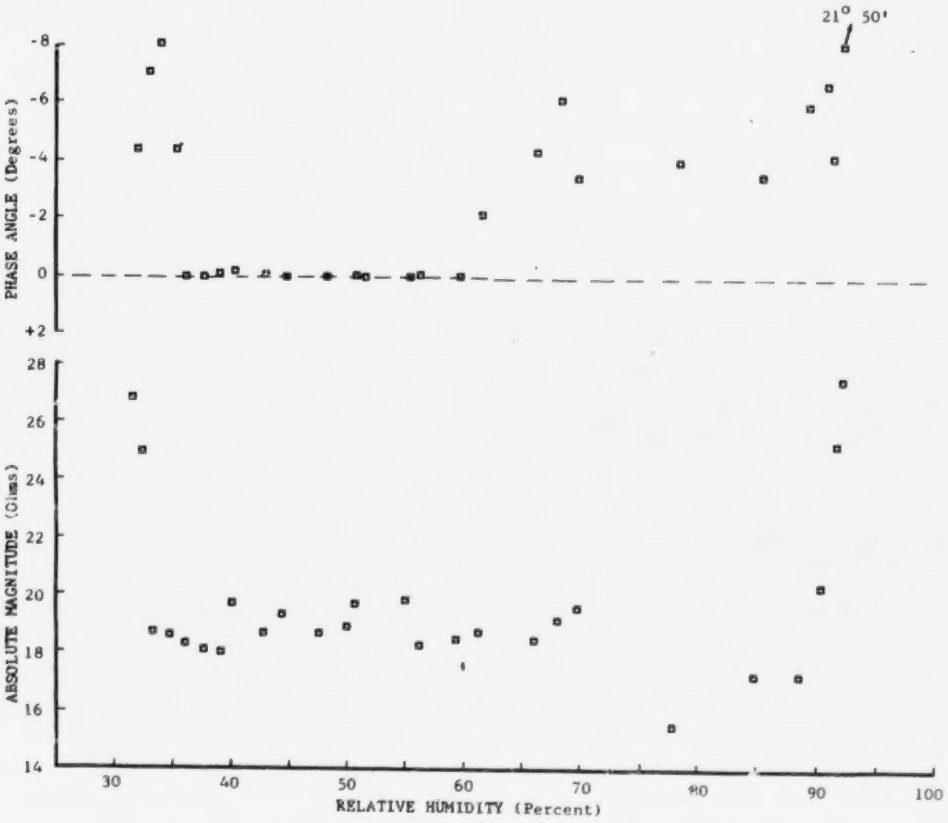


Figure 27. Absolute Magnitude and Phase Angle as a Function of Relative Humidity.

Summary

The cavity, as designed, can be made resonant between 370 and 390 MHz. Within this cavity, changes in impedance have been measured and related to relative humidity, placement of the monkey's head, and the head holder. Air with a relative humidity of less than 32 percent and constant temperature of 29° C is correlated with very rapid changes, while relative humidities above 90 percent (with temperature constant at 29° C) produce condensation on the antenna probe and radical changes.

It is conceivable that some of the changes in phase angle and absolute value of impedance observed in intermediate levels of relative humidity may also have resulted from condensation on the antenna probe. Obviously, the cerebral effects in the monkey are more consistent and replicable in relative humidity range of 40 percent to 60 percent at 29° C.

These differences in the reflected impedance with the monkey's head in the 0° and 30° elevation position indicate that the power in the real resistive component (component contributory to temperature changes) is greater at the 30° position than in the 0° position. These findings tend to confirm the earlier observations that greatest intracerebral power levels are obtained with the head elevated at 30° (with reference to the horizontal) and facing the antenna. These findings suggest that when the primate head is in an RF field a non-isotropic condition of power density distribution exists in the brain. These power levels vary significantly as the orientation of the head (in relationship to the field) is changed. It seems likely that such abnormal intracerebral power distributions could affect coincident or subsequent behavior which might be characteristic for the given head orientation at the time of exposure.

Future Course of Project

In a carefully designed protocol, the relationship of cerebral power levels to cerebral temperatures, EEG characteristics and behavior will be investigated. It is hoped that these experiments will yield information on the nature of RF effects on cerebral activity and to an understanding of the mechanism of action of microwave radiation effects, particularly at low levels of microwave radiation exposure.

METABOLIC STUDIES SECTION

Dr. DeWitt G. Hazzard, Chief

The Metabolic Studies Section evaluates the biological effects of several types of radiation, e.g., high and low energy X rays, microwaves, and ultrasound. The studies summarized in this report examine:

1) Direct effects of irradiation on DNA-RNA synthesis,
2) Effects of irradiation on immunological processes,
3) Effects of irradiation on enzyme systems,
4) Effects of irradiation on protein synthesis, and
5) Effects of irradiation on hematological and erythropoietic systems.

These studies are being conducted at the molecular, subcellular, cellular, organ, and whole animal levels. They involve effects of irradiation during the in utero, neonatal, juvenile, and adult stages of development and include both immediate and delayed responses. Other studies which are in progress involve effects of microwave and X-ray irradiations on the developing nervous system and effects of ultrasound on cellular function.

A. STUDIES ON THE EFFECT OF 2450 MHz MICROWAVES ON HUMAN IMMUNOGLOBULIN G[1]

Gopal P. Kamat, David E. Janes

Changes in the paper electrophoretic pattern and increase in the antigenic reactivity of human gamma globulin (HGG) have been reported for HGG solutions irradiated at specific frequencies within the 10-200 MHz range at 37.5° C.[2] Changes in the solubility of bovine gamma globulin (BGG) have been reported for irradiation at 13.1 MHz but not at 13.12 MHz at 25° C.[3] The simplest effect accounting for the changes observed in HGG and BGG is aggregate formation. Summarized here are the results of a series of experiments performed to determine if similar effects could be produced in solutions of human immunoglobulin G (IgG) microwave-irradiated at 2450 MHz.

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[1] A detailed paper will be published in the Proceedings of the Symposium on the Biological Effects and Health Implications of Microwave Radiation held in Richmond, Virginia, September 17-19, 1969.

[2] Bach, S. A., Luzzio, A. J., and Brownell, A. S.: Effect of Radio Frequency Energy on Human Gamma Globulin, Proc. Tri-Service Conf. on Biological Effects of Microwave Radiation, p. 117-133, 1961.

Human IgG was isolated from HGG (Cohn Fraction II, Pentex Inc.) by diethylaminoethyl (DEAE) cellulose chromatography.[4] IgG samples were suspended in air 16 cm in front of an open microwave oven (Amana Radar range, Model RR-1)[5]. Irradiations were performed at 2450 MHz with pulsed waves. The average temperature of the samples was measured with a nylon-coated thermistor probe (Series 423, Yellow Springs Instrument Co.). The samples were stirred with thermistor probe before recording the temperature and the probe was removed during irradiation.

Two IgG samples were irradiated and their elution profiles on Sepharose 4B columns compared with those of unirradiated IgG and with IgG which had been heated in a water bath at 50° C for 30 minutes. Both samples were irradiated intermittently over a period of about 30 minutes for a total irradiation time of 8 minutes. During irradiation, one of the samples was cooled with a stream of cold nitrogen. Fig. 28 shows the irradiation time and temperature profile of the uncooled sample. With the indicated pulse duration and repetition rate, temperature rose to 50° C within the first 6 minutes and dropped to about 40° C after 25 minutes. Fig. 29 shows the irradiation time and temperature profile of the nitrogen cooled sample. The temperature of the cooled sample decrease to about 5° C after 25 minutes.

The elution profiles of the irradiated, unirradiated and water bath heated samples are shown in Fig. 30. Because of the small variability in the position of the eluate peaks, sedimentation coefficient values ($S_{20,w}$) for the material in the main chromatographic peaks were determined. The results are shown in Table 15 and the sedimentation patterns are shown in Fig. 31. The similarity of the elution profiles and of the $S_{20,w}$ values suggests that no gross changes occur in the structure of the soluble fraction of human IgG.

Samples of IgG purified by DEAE cellulose chromatography were irradiated with a microwave oven for 8.5, 7.0, and 5.5 minutes fractionated respectively over 30, 20, and 10 minute intervals. The samples were irradiated with the same pulse duration and distribution as was used

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[3] U. S. Army Contract No. DA 18-108-CML-7061 (Melpar Inc., Research Division, Falls Church, Virginia). Final report on Biological Effects of R-F on Macromolecules, August, 1962.

[4] Levy, H. B. and Sober, H. A., Proc. Soc. Exp. Biol. Med. 103: 250-252, 1960.

[5] Modified to operate with the door open.

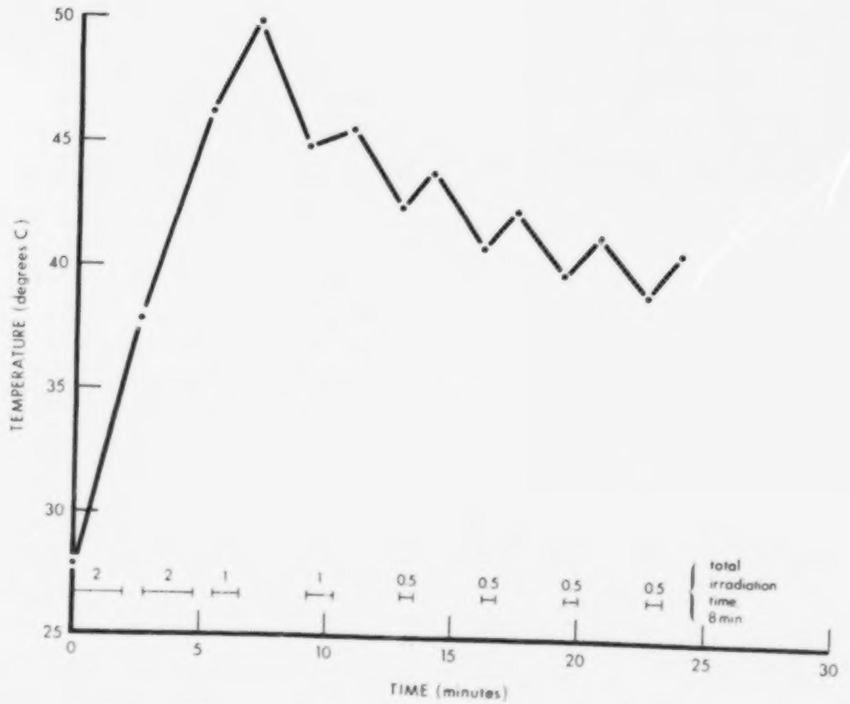


Figure 28. Temperature of Microwave Irradiated Human IgG Solution. IgG solution in a polystyrene tube was suspended outside the oven in air 16 cm in front of the oven cavity. The duration of microwave pulses is indicated by the horizontal cross bars.

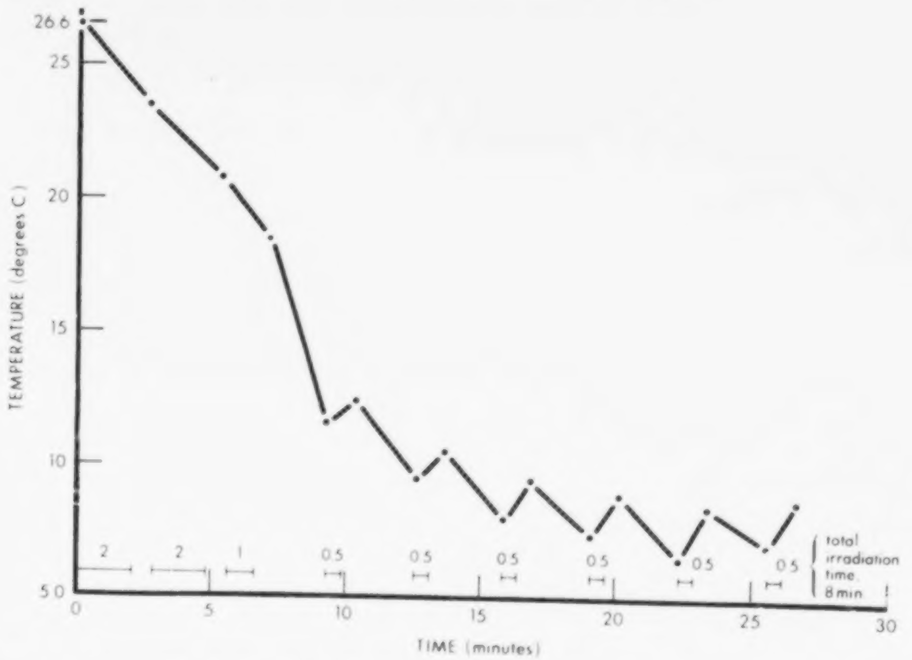


Figure 29. Temperature of Microwave Irradiated Human IgG Solution Cooled with Nitrogen. Irradiation conditions were as described in Figure 28.

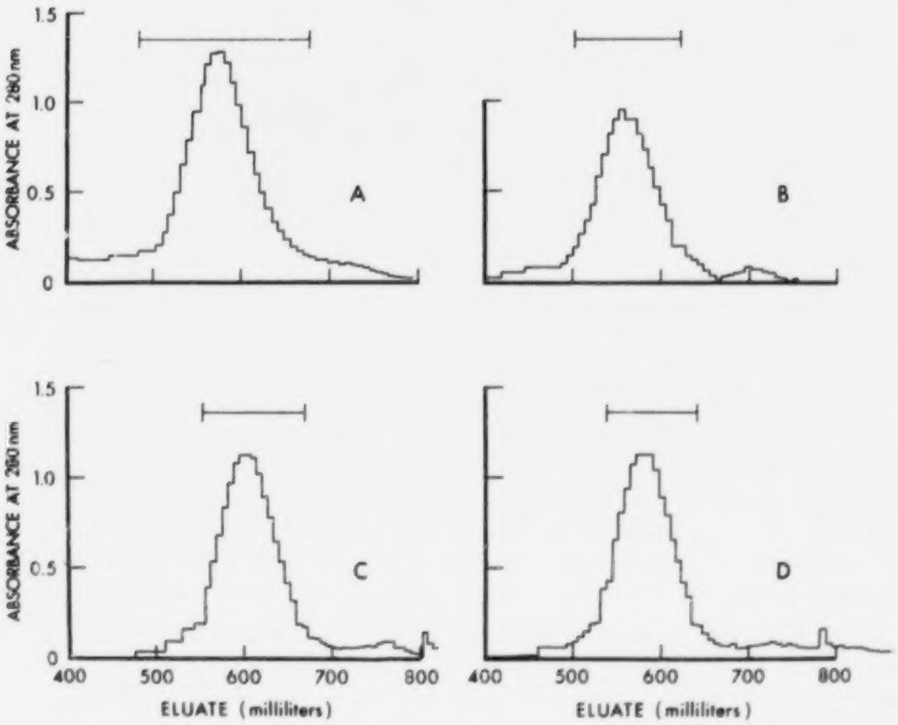


Figure 30. Fractionation of Human IgG on Sepharose 4B. (A) No irradiation, (B) Irradiated as described in Figure 28, (C) Irradiated as described in Figure 29, (D) Heated for 30 min in 50° C water bath. All samples were composites from DEAE-cellulose chromatography run No. A, B, and C. Concentration of each sample was 20.0 mg/ml in 0.0175 M phosphate buffer, pH 6.3. Eluting buffer: 0.01 M phosphate buffer, pH 7.6 containing 0.15 M NaCl. Column size: 42.0 x 5.0 cm. The sedimentation coefficients for the fractions under the cross bars are given in Table 13 and their ultracentrifugation patterns are shown in Figure 31.

TABLE 15. SEDIMENTATION COEFFICIENTS FOR HUMAN IMMUNOGLOBULIN G

Treatment	Protein Conc. (mg/ml)	$S_{20,w} \times 10^{13}$
A[a] Untreated	4.2	6.3
B Irradiated at 2450 MHz with rise in temperature	3.9	6.1
C Irradiated at 2450 MHz with no rise in temperature	6.5	6.0
D Heated in water bath (50° C, 30 min)	5.9	5.7

a. Letters correspond to samples in Figure 30.

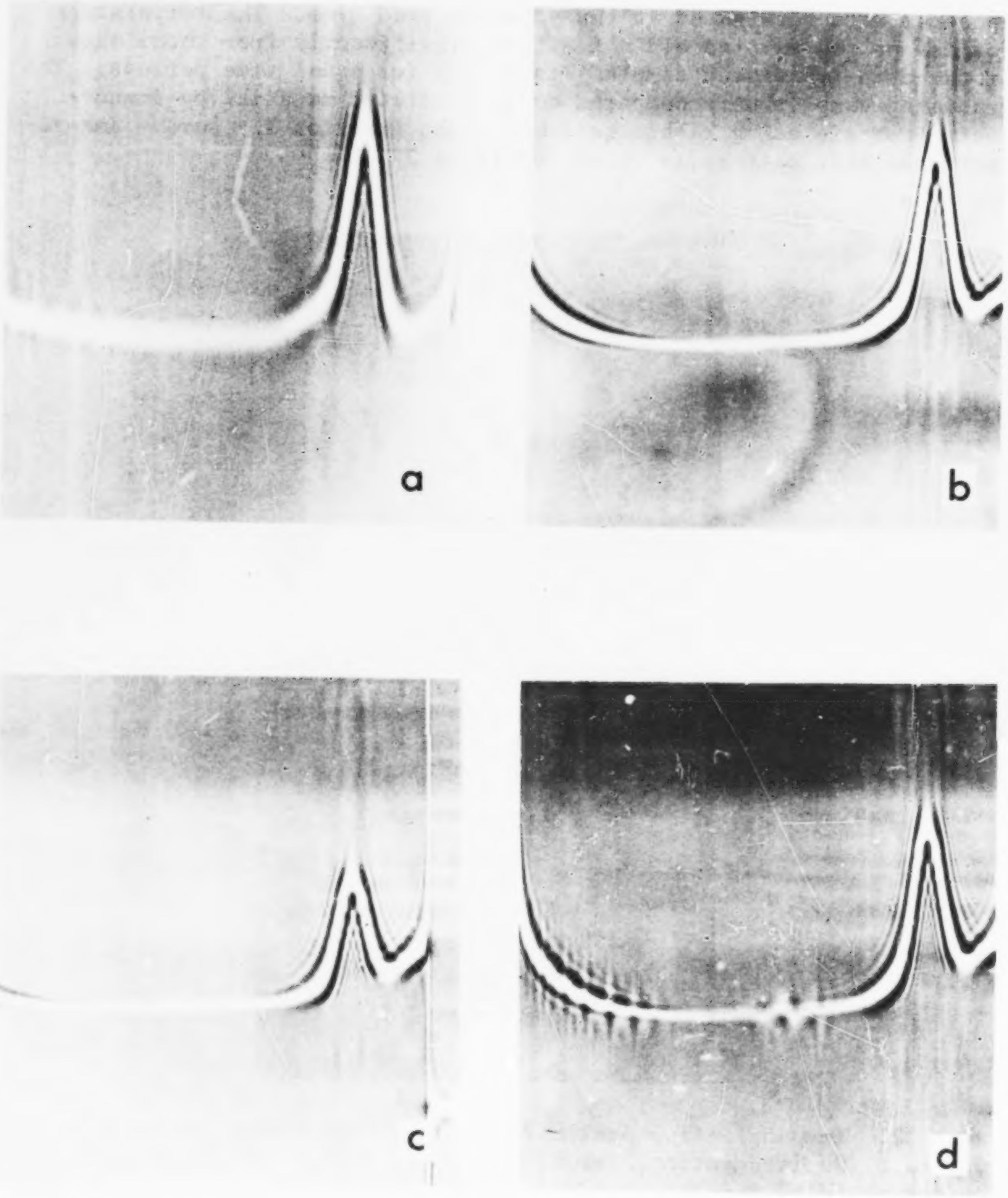


Figure 31. Analytical Ultracentrifugation Patterns of the Sepharose 4B Fractions Shown in Figure 30. Speed: 59,800 rpm, temperature: 20° C, solvent: 0.01 M phosphate buffer pH 7.6 containing 0.15 M NaCl. Photographs shown are those that were taken 16 minutes after attaining 59,800 rpm.

in irradiating the nitrogen cooled sample (Figure 29) except that the exposures were terminated at the given elapsed time. The temperature profiles of the samples did not differ significantly from those shown for the oven-irradiated sample (Figure 28) for equal time periods. The irradiated samples were compared to unirradiated material by immunoelectrophoresis using rabbit anti-HGG (Cohn Fraction II) sera. Immunoelectrophoretic pattern is shown in Figure 32.

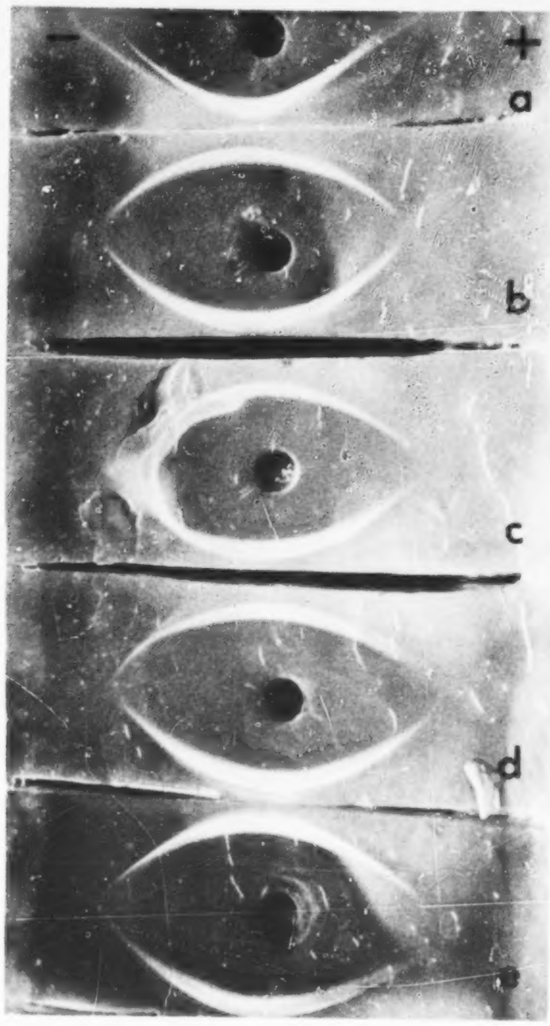


Figure 32. Immunoelectrophoretic Patterns of Microwave Irradiated (2450 MHz) IgG. (a,b) No irradiation, (c) 8.5 minutes irradiation fractionated over 30 minutes, (d) 7 minutes irradiation fractionated over 20 minutes, (e) 5.5 minutes irradiation fractionated over 10 minutes. The irradiation conditions are as described in Figure 29. The source was pulsed as shown in Figure 30 except that the exposures were terminated at the given elapsed times. The temperature profiles were similar to those given in Figure 29 over equal time intervals. All samples were from DEAE cellulose chromatography run No. C. The concentration of the samples was about 20 mg/ml in 0.0175 M phosphate buffer pH 6.3. Antisera: Rabbit anti-Cohn Fraction II.

The results show that in addition to the strong precipitin line given by IgG there was an additional line in the unirradiated material which indicates the presence of some impurity. Additional faint precipitin lines were seen in the patterns of the irradiated IgG. One additional faint precipitin line was observed for IgG irradiated for a total irradiation time of 10 minutes at 50° C and two additional faint precipitin lines were observed for IgG samples irradiated for total irradiation times of 20 minutes at 50° C and 30 minutes at 50° C. The faint additional precipitin lines seen in the immunoelectrophoretic patterns of IgG irradiated with the oven may be due to impurities.

Samples of IgG were heated in a water bath for 30 minutes at temperatures of 40, 50, and 60° C, respectively. The immunoelectrophoretic patterns of the heated and the unheated samples are shown in Figure 33. The unheated sample shows the splitting that is correlated with the presence of lower molecular weight material but appears to be free of the impurity seen in the earlier preparation (see Figure 32). Additional faint precipitin lines were not observed in the patterns of the water bath heated material.

Four human IgG samples were irradiated with a microwave generator for 7.0, 8.0, 9.0 and 10.5 minutes fractionated, respectively, over 10.0, 20.0, 30.0 and 40.0 minutes. The temperature profile of the IgG sample irradiated for 10.5 minutes fractionated over a time interval of 40.0 minutes is shown in Fig. 34. The irradiations of 7.0, 8.0 and 9.0 minutes were performed with the same pulse duration and distribution as shown in Figure 34 except that the exposures were terminated at given elapsed times of 10.0, 20.0 and 30.0 minutes respectively. The temperature profiles of these samples did not differ significantly from that shown in Figure 34 for equal time periods. The power density measured in air along the principal axis with 9.1 cm dipole and power meter (model no. 413C, Hewlett Packard Inc.) was 450 mW/cm (± 10%) at 53 cm in front of the horn.

The immunoelectrophoretic patterns of the irradiated and the unirradiated samples using rabbit antiserum against irradiated HGG (Cohn Fraction II) are shown in Figure 35. The results show only one precipitin line, migrating towards the cathode, for the irradiated and the unirradiated samples. The splitting of the single IgG line did not occur in these samples. A sample of human IgG was irradiated with the microwave generator for 10.5 minutes fractionated over 40.0 minutes. The pulse duration and distribution were the same and the temperature profile essentially the same as those shown in Fig. 34. The insoluble aggregates from the irradiated IgG cooled to room temperature were isolated by centrifuging at 105,000 g at 4° C for 90 minutes. The pellet containing the aggregate was resuspended in 0.1 M barbitone buffer which was acidified with HCl to a pH 5.0.

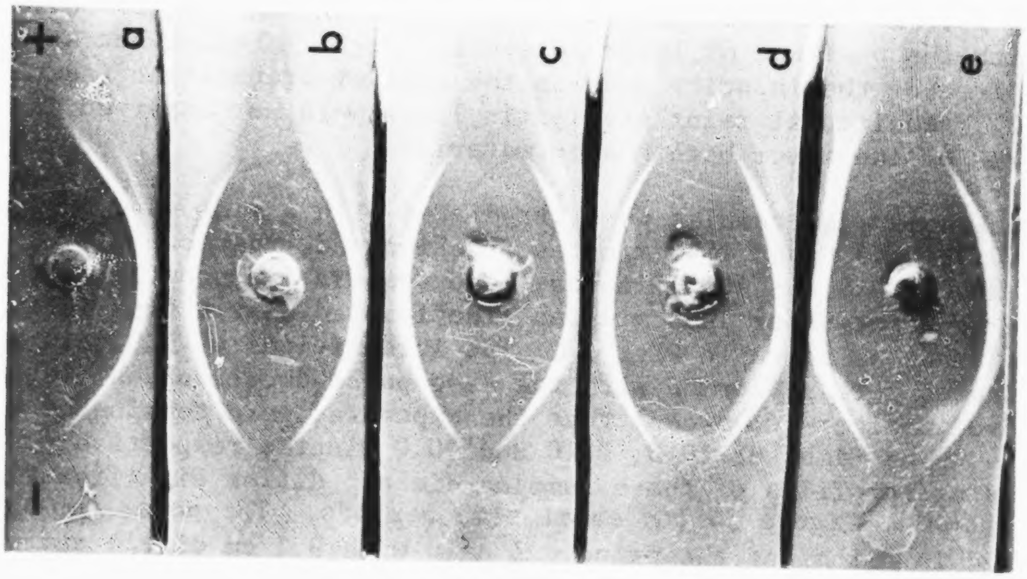


Figure 33. Immunoelectrophoretic Patterns of Water-Bath-Heated Human IgG. (a,b) No irradiation, (c) heated at 60° C for 30 min, (d) heated at 50° C for 30 min, (e) heated at 40° C for 30 min. All samples were from DEAE cellulose chromatography run No. E. The concentration of the samples was 20.0 mg/ml in 0.0175 M phosphate buffer, pH 6.3. Antisera: Rabbit Cohn Fraction II.

The immunoelectrophoretic pattern of the unfractionated irradiated, unirradiated IgG, the aggregate, and the supernatant after aggregate removal are shown in Figure 36. A single precipitin line is shown migrating toward the cathode, both for the unfractionated irradiated and unirradiated IgG samples. The splitting of the IgG line did not occur in these samples. The electrophoretic mobility of the supernatant separated from the irradiated IgG was similar to the mobility of the unirradiated IgG, and the mobility of the aggregate was less than the mobility of the unirradiated IgG. The aggregates were insoluble, i.e.,

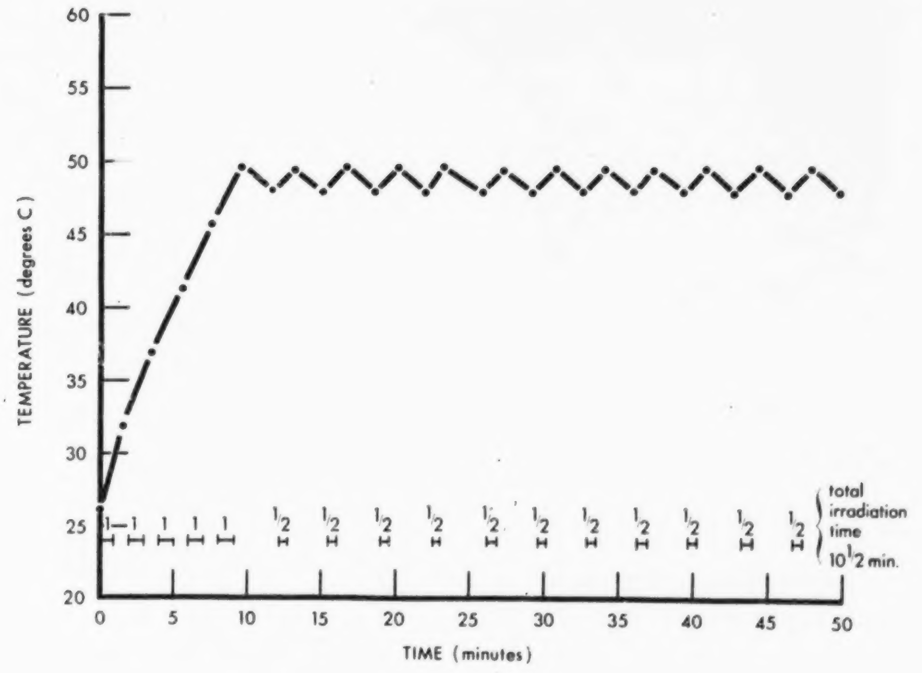


Figure 34. Temperature of Microwave Irradiated Human IgG Solution. The IgG samples in polystyrene tubes were placed in a polystyrene foam block 53.0 cm in front of the horn of a microwave generator. The duration of microwave pulses is indicated by the horizontal cross bars.

a precipitin line for the aggregated material is seen only when the aggregates are isolated by ultracentrifugation, redissolved as described above and then subjected to immunoelectrophoresis.

The results of this study indicate that there is no qualitative difference between the effects observed when IgG is irradiated either with microwaves or heated with a water bath. Microwave heating of IgG solutions causes the formation of insoluble aggregates. There are no gross changes produced in the structure of the soluble fraction of human IgG when irradiated either with microwaves or heated with a water bath. The faint additional precipitin lines seen in some samples of IgG may be due to impurities.

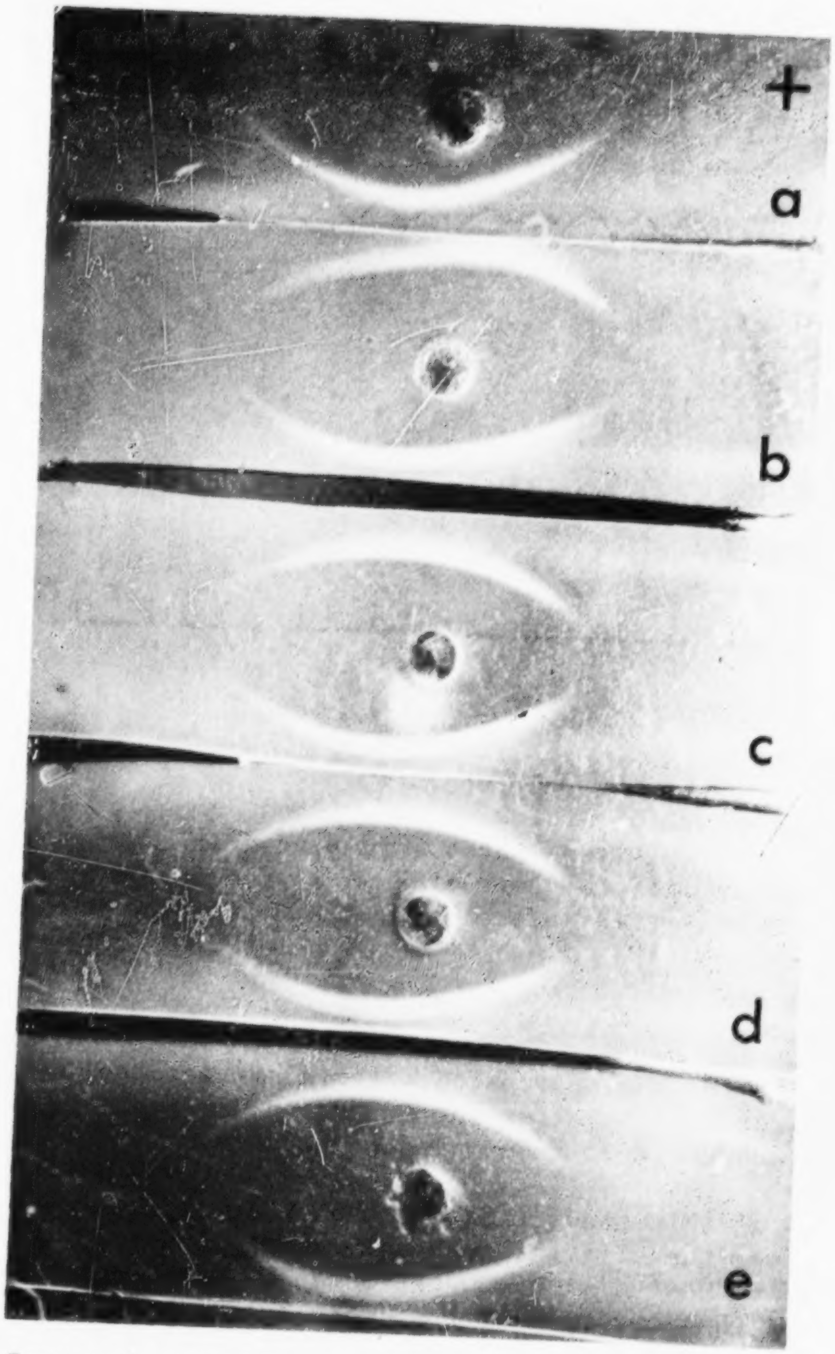


Figure 35. Immunoelectrophoretic Pattern of Microwave Irradiated Human IgG. (a) No irradiation, (b) 7.0 minutes irradiation fractionated over 10 min, (c) 8.0 minutes irradiation fractionated over 20 min, (d) 9.0 min irradiation fractionated over 30 min, (e) 10.5 minutes irradiation fractionated over 40 min. The microwave source was pulsed as shown in Figure 34 over equal time intervals. All samples were from DEAE cellulose chromatography run No. F. The concentration of the sample was 20.0 mg/ml in 0.0175 M phosphate buffer, pH 6.3. Antisera: Rabbit anti-irradiated Cohn Fraction II.

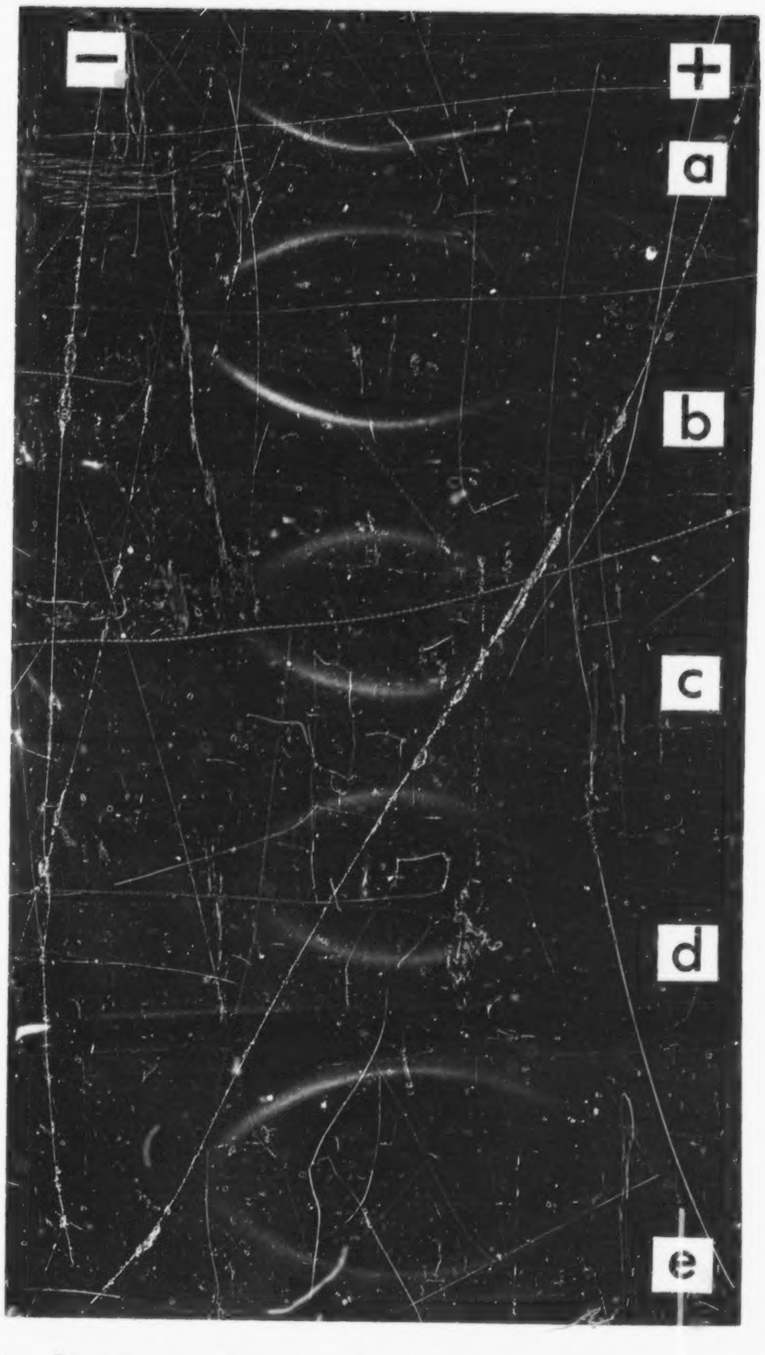
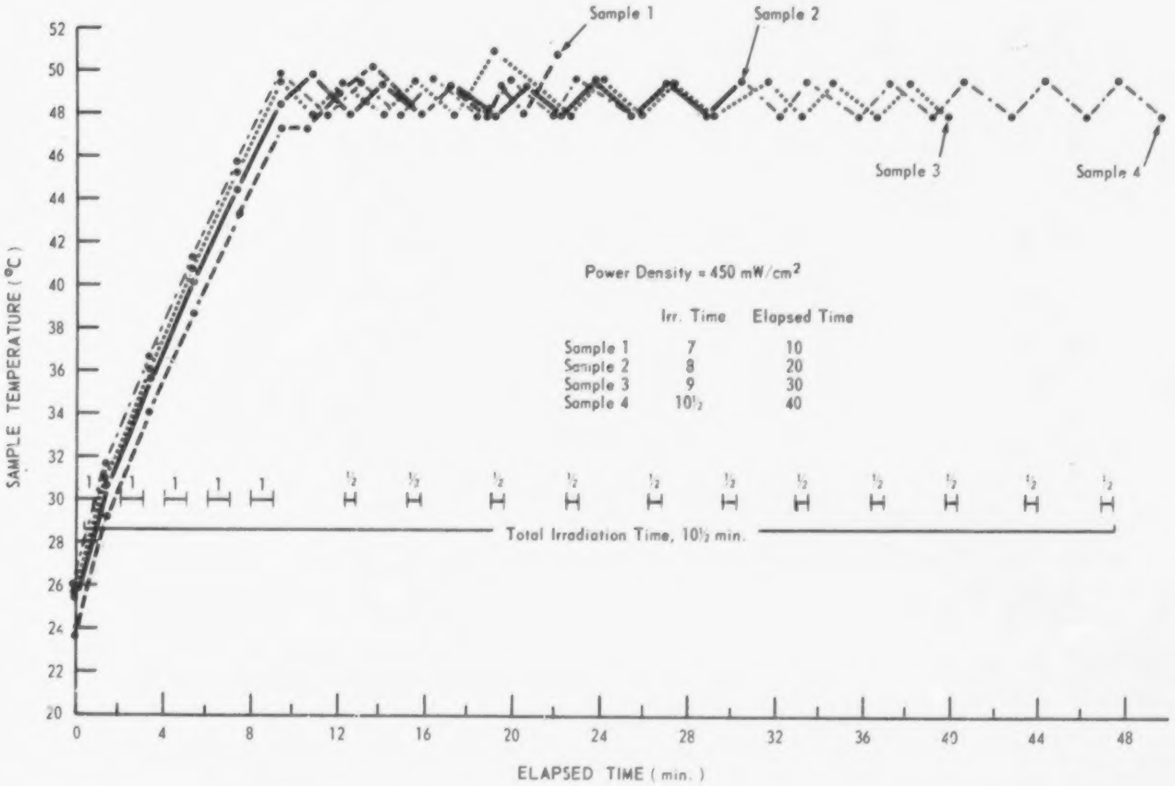


Figure 36. Immunoelectrophoretic Pattern of Microwave Irradiated Human IgG. (a) No irradiation, (b) 10.5 minutes irradiation fractionated over 40 minutes, (c,d) aggregate, (e) supernatant after aggregate removal. The microwave source was pulsed as shown in Figure 34 and the temperature profiles were the same as those in Figure 34. All samples were from DEAE cellulose chromatography run No. G. The concentration of the sample was 20.0 mg/ml in 0.0175 M phosphate buffer, pH 6.3. Antiserum: Rabbit anti-Cohn Fraction II. Aggregate was isolated by centrifuging the irradiated IgG at 103,000 g at 4° C for 90 minutes in Spinco rotor 40.3.

B. STUDIES ON THE BIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF 2450 MHz MICROWAVE IRRADIATED HUMAN IMMUNOGLOBULIN G (IgG)

Gopal P. Kamat

Samples of human IgG, 3.0 ml, were irradiated one at a time with 2450 MHz microwaves from a generator with a horn. The samples were irradiated for 10 min, 20 min, 30 min and 40 min after attaining the temperature of 50° C by operating the generator in cycles. The curves obtained by plotting the elapsed time in minutes against the sample temperature were similar (Fig. 37). It is assumed from these results that the microwave irradiation for the four samples was performed under similar conditions. An attempt to irradiate the samples all together by placing them vertical or parallel to the direction of propagation of microwaves was unsuccessful because during the same irradiation period the four samples were heated to different temperatures.



	Irr. Time	Elapsed Time
Sample 1	7	10
Sample 2	8	20
Sample 3	9	30
Sample 4	10½	40

Figure 37. Temperature Profiles of Microwave Irradiated Human IgG for Various Time Intervals. Elapsed time in table represents time after attaining 50° C.

Passive Cutaneous Anaphylaxis[1]

Ten dilutions of the high titer immune serum prepared in rabbits against microwave-irradiated Cohn Fraction II were injected intradermally in an albino guinea pig and the animal was challenged 4 hours later by the intravenous injection of a mixture of Evans Blue and microwave-irradiated human IgG. The results showed that all dilutions of the antiserum gave a positive direct PCA reaction (Table 16). The direct PCA reaction was negative in the guinea pig which was injected intradermally with the same dilutions of either immune sera prepared in rabbits against microwave-irradiated Cohn Fraction II or unirradiated Cohn Fraction II and challenged 4 hours later by intravenous injection of unirradiated human IgG.

TABLE 16. PASSIVE CUTANEOUS ANAPHYLAXIS USING TEN DILUTIONS OF ANTISERUM AGAINST MICROWAVE-IRRADIATED COHN FRACTION II AND MICROWAVE-IRRADIATED HUMAN IgG AS ANTIGEN

Antiserum Dilution	Reaction Intensity	Diameter of Bluing, mm
1:1	***	4
1:2	***	4
1:3	**	3
1:4	**	3
1:5	**	3
1:6	*	2
1:7	*	2
1:8	*	2
1:9	*	2
1:10	*	2

Reversed Passive Cutaneous Anaphylaxis[1]

An albino guinea pig was injected intradermally with different dilutions of either microwave-irradiated human IgG or unirradiated human IgG and the saline control in the same animal and challenged 4 hours later by an intravenous injection of a mixture of Evans Blue and the high titer immune serum against microwave-irradiated Cohn Fraction II. The results showed that the microwave irradiated human IgG showed a positive reversed PCA reaction while the unirradiated human IgG showed a negative reversed PCA reaction (Table 17).

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[1] Zoltan Ovary, Progr. Allergy 5:459-508, 1958.

TABLE 17. REVERSED PASSIVE CUTANEOUS ANAPHYLAXIS USING MICROWAVE-IRRADIATED OR UNIRRADIATED HUMAN IgG AS ANTIGEN AND ANTISERUM AGAINST MICROWAVE-IRRADIATED COHN FRACTION II

Unirradiated		Irradiated		
Antigen Conc., µg	Reaction	Antigen Conc., µg	Reaction Intensity	Diameter of Bluing, mm
400	-	400	**	2
800	-	800	***	4
1200	-	1200	***	4
1600	-	1600	***	4
Saline control	-	Saline control	-	

Quantitative Antigen-Antibody Precipitation Reaction

The precipitation reaction between the unirradiated or microwave-irradiated human IgG and the immune serum against the microwave irradiated or unirradiated Cohn Fraction II was examined by adding a constant amount of the antibody to different amounts of the antigen. The total protein in the antigen-antibody precipitate and in the antigen was measured quantitatively by phenol method as modified by Doughaday[2]. The results showed that the precipitation of the microwave-irradiated antigen with its homologous antibody was at least 50% less than the precipitation of the native antigen with its homologous antibody (Fig. 38, 39).

The precipitate of the irradiated antigen and its homologous antibody was at least 70% less soluble in excess antigen than the precipitate of the native antigen with its homologous antibody. The precipitation of either the irradiated antigen and the antibody against unirradiated Cohn Fraction II or the unirradiated antigen and the antibody against irradiated Cohn Fraction II was less than the precipitation of the native molecule with its homologous antibody and was more than the precipitation of the irradiated antigen with its homologous antibody.

Susceptibility of Disulphide Bonds in Microwave Irradiated and Native Human IgG for Reduction with 2-Mercaptoethanol

In this study, the thiol groups (SH^-) were estimated quantitatively by using Ellman's reagent (5,5'-dithio bis-2 nitrobenzoic acid) as

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[2] Doughaday, W.H. et al., J. Lab. Chem. Med. 39:663, 1952.

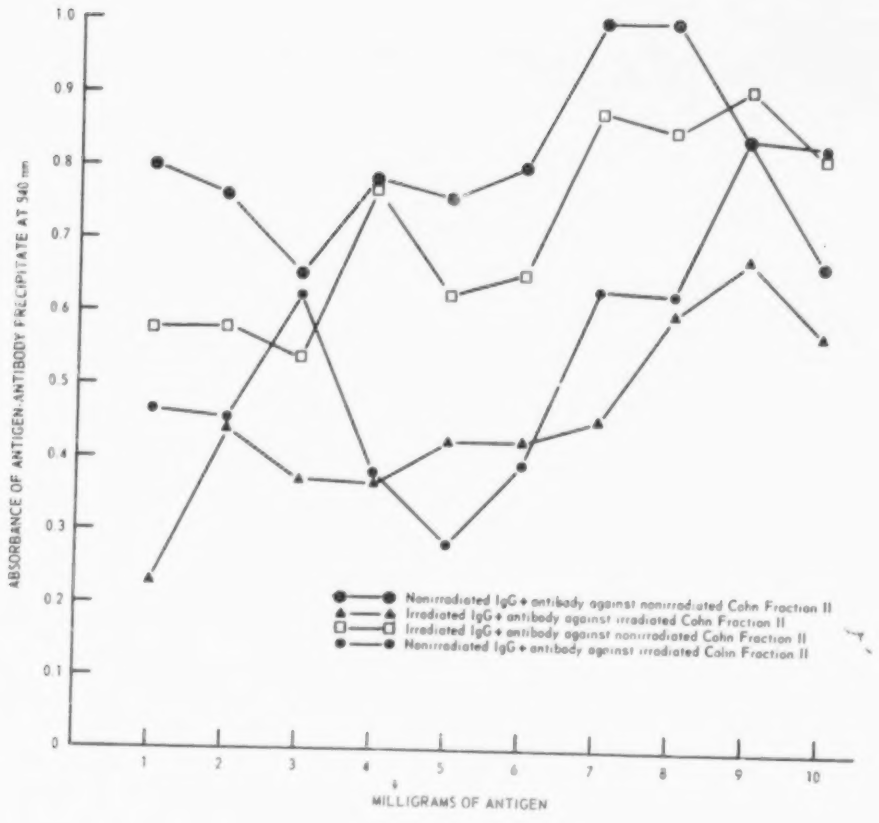

Figure 38. Quantitative Antigen-Antibody Precipitation Reaction Using Irradiated or Unirradiated Human IgG and Antiserum Against Irradiated or Unirradiated Cohn Fraction II.

described by Diez et al.[3,4] The results showed that the disulphide bonds in microwave-irradiated human IgG are at least 35% more susceptible for reduction with 2 mercaptoethanol than the disulphide bonds in native human IgG.

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[3] G. L. Ellman, Arch. Biochem. Biophys. 82:70, 1959.
[4] M. J. F. Diez et al., Arch. Biochem. Biophys. 107:449, 1964.

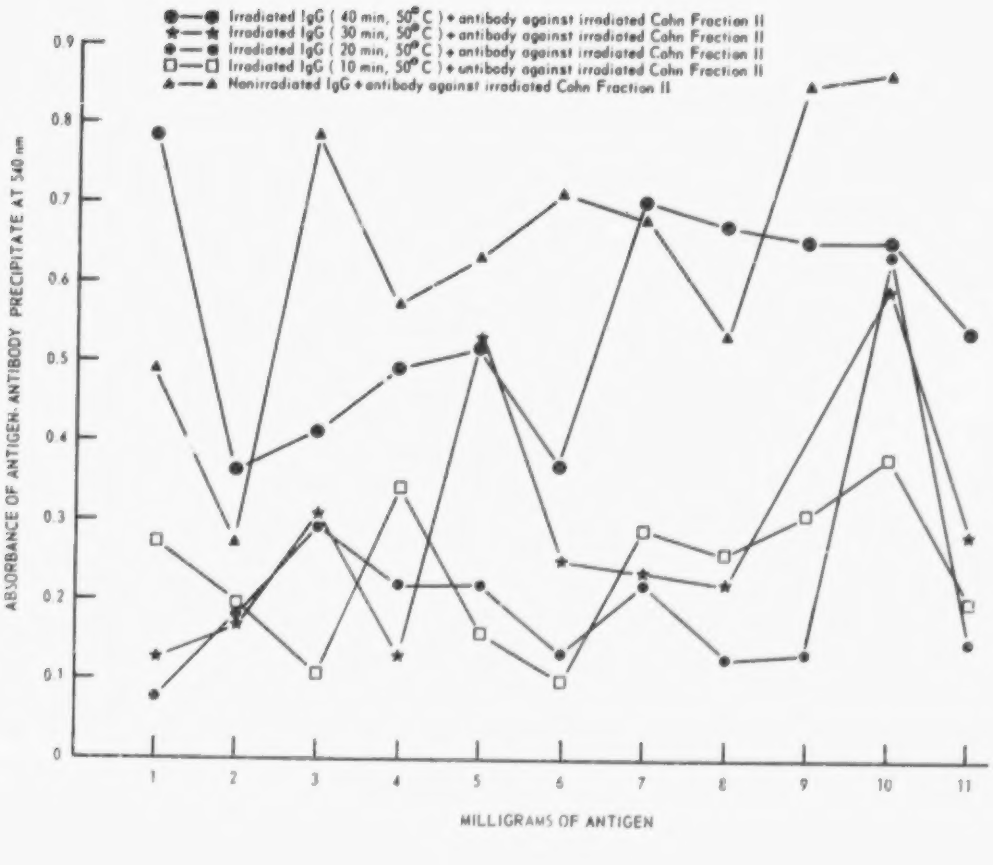

Figure 39. Quantitative Antigen-Antibody Precipitation Reaction Using Irradiated Human IgG at 10, 20, 30, and 40 minutes at 50° C as Antigen and Antibody Against Irradiated Cohn Fraction II.

C. THERMAL INACTIVATION OF RIBONUCLEASE

David E. Janes

The extensively studied thermal denaturation of ribonuclease[1] gives a model system which can be used to study source specific thermal effects such as the anisotropic temperature distribution which may result from microwave irradiation. The change in ultraviolet spectra accompanying denaturation is completely reversible in heating cycles where the temperature does not exceed the transition temperature, T_t, but is only partially reversible otherwise.[2] (T_t is the temperature at which half of the ribonuclease is reversibly denatured. It increases from 25° to 65° C as the pH is raised from 0.9 to 6.8). We show here that the irreversible spectral changes that occur when ribonuclease is heated at 80° C are paralleled by losses in enzyme activity.

The assay for enzyme activity is modeled after that of Richards[3] as modified by Crook et al.[4] and depends on the difference between the absorbance of the substrate, cytidine 2':3'-phosphate, and the product, cytidine 3'-phosphate, at 286 nm. The data were analyzed by assuming that the hydrolysis of the substrate was initially first order, i.e.,

$$-(dS/dt) = kES \tag{1}$$

$$(A_\infty - A_t) = (\varepsilon_p - \varepsilon_s)S \tag{2}$$

where k is a constant, E and S are respectively the enzyme and substrate concentrations, A_t is the absorbance at time t, A_∞ is the absorbance at complete reaction, and ε_p and ε_s are the molar extinction coefficients of product and substrate. Separating variables in Eq. (1), integrating, and substituting for S its value from Eq. (2) yields

$$\log(A_\infty - A_t) = kEt + \log(A_\infty - A_o) \tag{3}$$

Values of kE determined from plots of $\log(A_\infty - A_t)$ versus time, Figure 40, were plotted against enzyme concentration to give the calibration curve, Figure 41.

Ribonuclease samples (1.5 ml, C=1.8 g/1) were heated in thin walled plastic centrifuge tubes placed in an 80° C (±1°) water bath for 10 minutes and for 30 minutes. The samples were rapidly cooled to 25° C in an ice bath. The ultraviolet difference spectra and enzyme activities of the heated samples are compared to those of an unheated sample in

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[1] R. A. Scott and H. A. Scheraga, J. Amer. Chem. Soc., 85:3866, 1963.

[2] J. Hermans, Jr. and H. A. Scheraga, J. Amer. Chem. Soc., 83:3283, 1961.

[3] F. M. Richards, Compt-rend. Lab. Carlsberg, Ser. chim., 29:315, 1955.

[4] E. M. Crook, A. P. Mathias, and B. R. Rabin, Biochem. J., 74:234, 1960.

Figures 42 and 43. The concentration of active enzyme in the heated samples, determined with the aid of Figures 41 and 43, is plotted against the corresponding change in absorbance in Figure 44. The results in Figure 44 show that the irreversible changes in ultraviolet spectra that occur when ribonuclease is heated above T_t are paralleled by losses in enzyme activity. Ribonuclease loses 40% of its activity after 10 minutes and practically all of its activity after 30 minutes at 80° C.

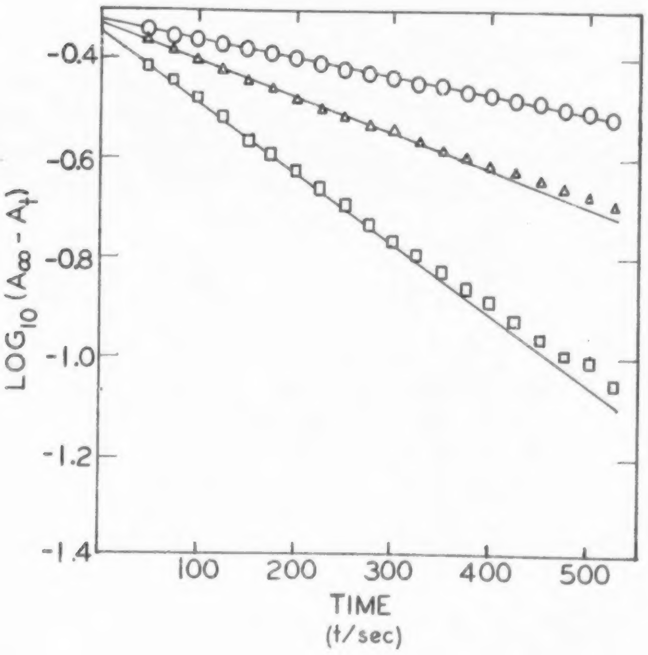

Figure 40. Examples of Plots Used to Determine the Apparent First Order Rate Constants kE. The substrate concentration was 0.1 g/l in Tris-HCl buffer, pH 7.1 and the enzyme concentrations in the assay reaction mixture were: ○, 5.6×10^{-3} g/l; △, 11.3×10^{-3} g/l; □, 24.3×10^{-3} g/l. The reaction was started by adding 10 μl of the enzyme sample to 3 ml of substrate solution.

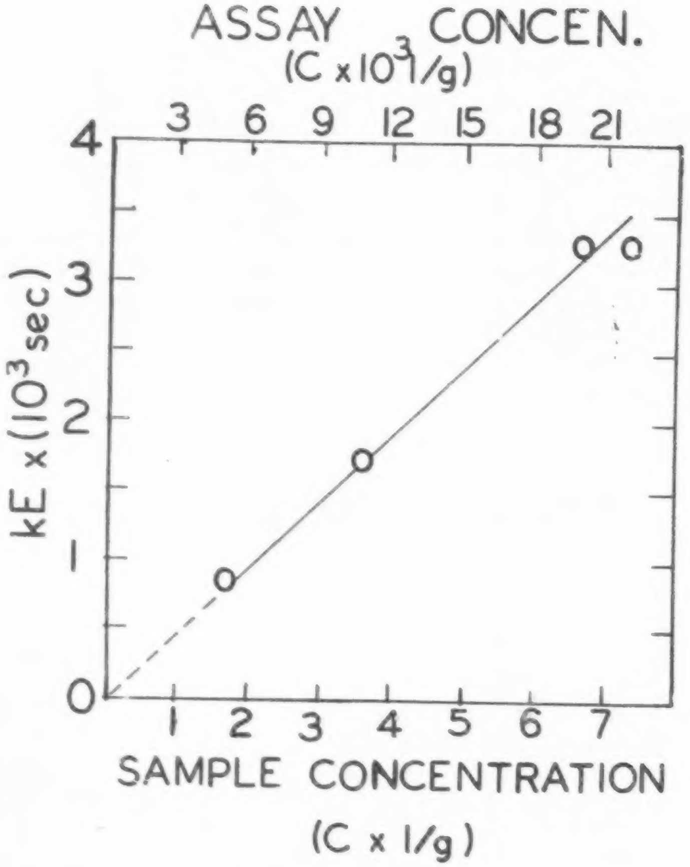

Figure 41. Plot of Apparent First Order Rate Constants kE Against Enzyme Concentration in the Reaction Mixture (upper scale) and in the Sample (lower scale).

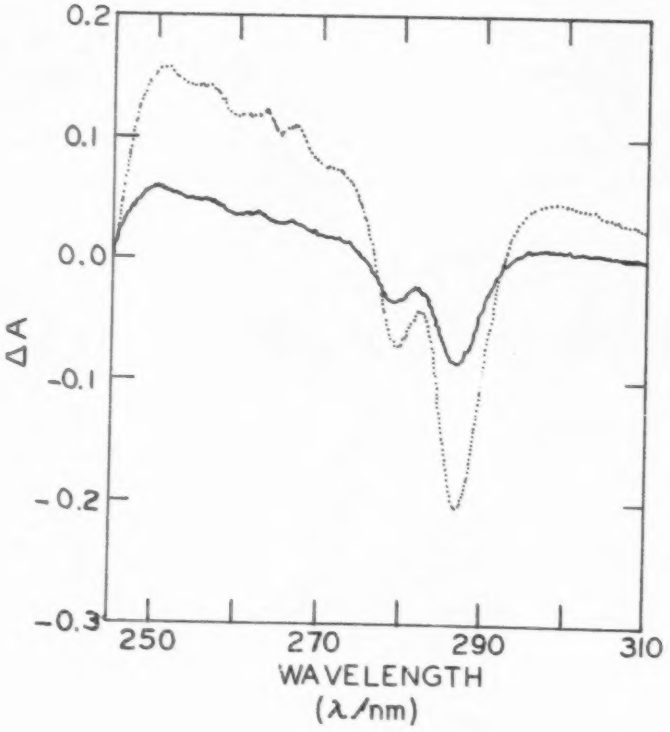

Figure 42. The Difference Spectra of Heat Denatured Ribonuclease when Compared with Unheated Ribonuclease. Enzyme Concentration 1.8 g/l in distilled water: ——, heated at 80° C for 10 minutes; ···, heated at 80° C for 30 minutes.

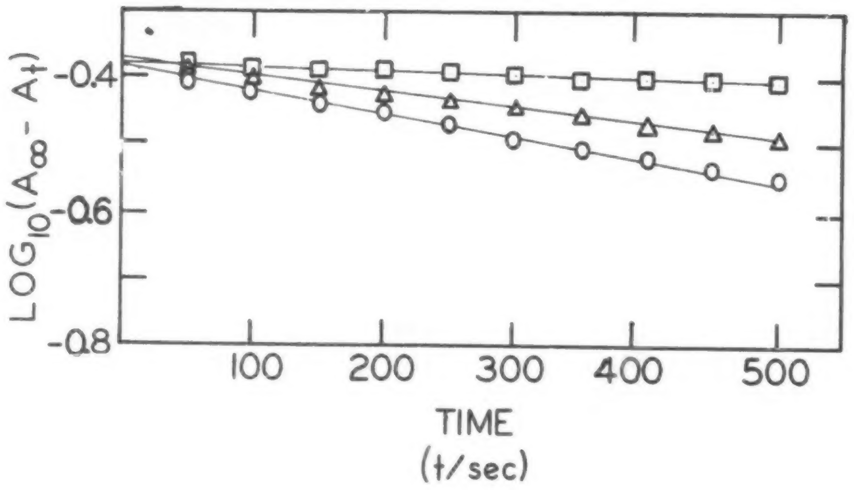

Figure 43. Plot Used to Determine Apparent First Order Rate Constants of Heat Denatured Ribonuclease. The substrate concentration was 0.1 g/l in Tris-HCl buffer, pH 7.1. The reaction was started by adding 10 μl of enzyme solution (C = 1.8 g/l) to 3 ml of substrate solution: ○, unheated; △, 80° C for 10 minutes; □, 80° C for 30 minutes.

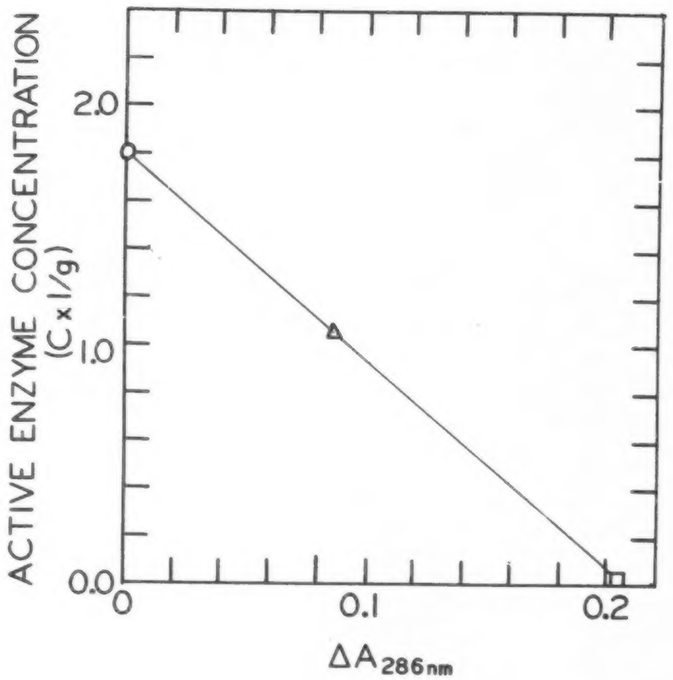

Figure 44. Plot of the Active Enzyme Concentration Remaining in Heat Denatured Ribonuclease Against the Corresponding Change in Absorbance at 286 nm from Figure 42: ○, unheated; △, 80° C for 10 minutes; □, 80° C for 30 minutes.

D. EFFECTS OF *IN UTERO* RADIATION ON THE PERIPHERAL BLOOD OF THE NEONATAL RAT[1]

DeWitt G. Hazzard, Roger A. Budd

X-irradiation was given *in utero* at 15 days gestation to Sprague-Dawley strain rats at an absorbed dose rate of 78 rads/min. The dams were injected intraperitoneally one day before irradiation either with endotoxin, colchicine, or saline. Hemopoietic changes in the peripheral blood were determined on newborn animals, one day after birth.

The effects of radiation alone were studied at doses of 0, 60, 120, 180, and 240 rads respectively. The irradiated rats showed a statistically significant ($P < 0.01$) linear decrease in body weight; the decrease, however, was less than that of irradiated animals receiving either endotoxin or colchicine (Fig.45).

The effects of drugs and radiation were tested at the 120, 180, and 240 levels using a balanced 3 x 3 factorial analysis. Radiation alone caused a statistically significant ($P < 0.01$) linear decrease in white blood cells. Irradiated animals receiving endotoxin had higher white blood cell counts than animals receiving radiation alone (Fig. 46).

Radiation alone caused no significant difference in red blood cells or hematocrit (Fig. 47,48). The groups receiving radiation and endotoxin had higher red blood cell counts and hematocrit than the groups receiving radiation alone. Nucleated red blood cells showed no appreciable changes resulting from radiation alone or in combination with

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[1] A detailed report of this study will be published in the Proceedings of the Ninth Annual Hanford Biology Symposium, May 1969.

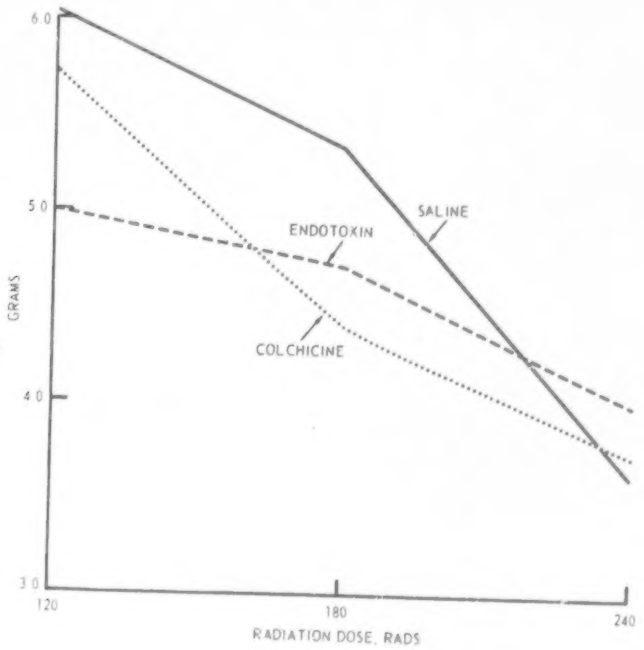

Figure 45. Effect of Radiation Dose and Drugs on Body Weight.

endotoxin or colchicine (Figure 49).

In conclusion, fetal leukocytes appear to be very radiosensitive, while erythrocytes are relatively radioresistant. Pretreatment with endotoxin resulted in an increased leukocyte response after birth and a possible increase in survival rate. Colchicine was not able to offer similar radioprotection to the fetal hematological system.

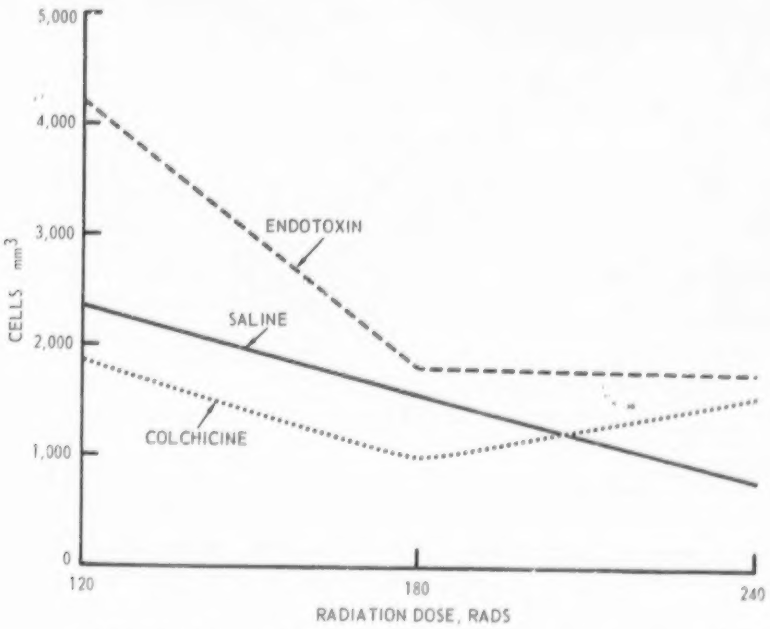

Figure 46. Effect of Radiation Dose and Drugs on White Blood Cell Count.

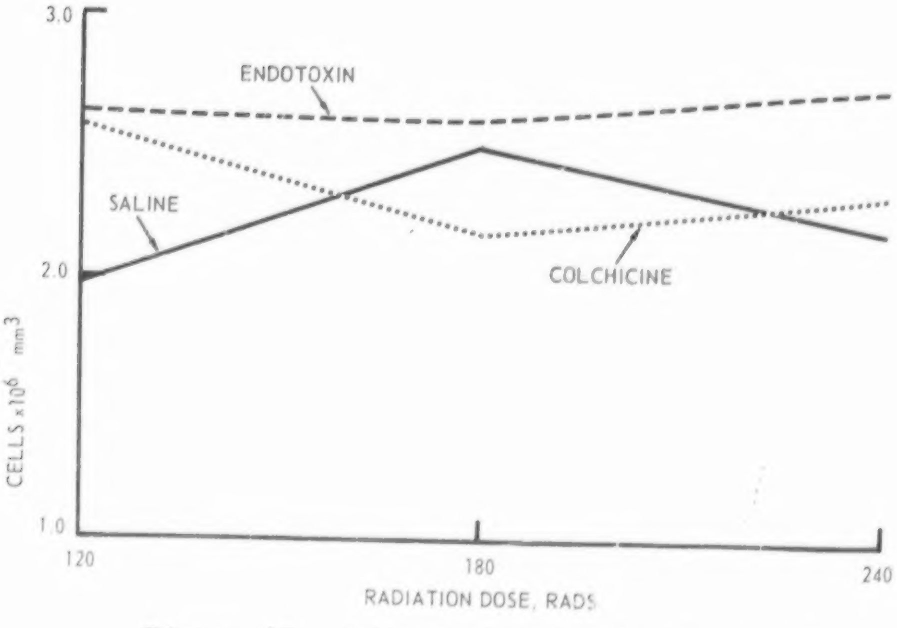

Figure 47. Effect of Radiation Dose and Drugs on Total Red Blood Cell Count.

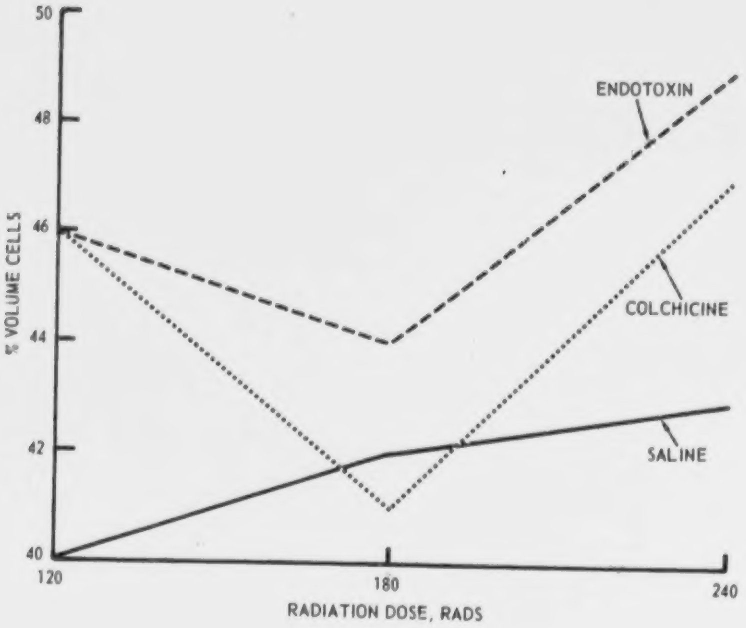

Figure 48. Effect of Radiation Dose and Drugs on Hematocrit Values.

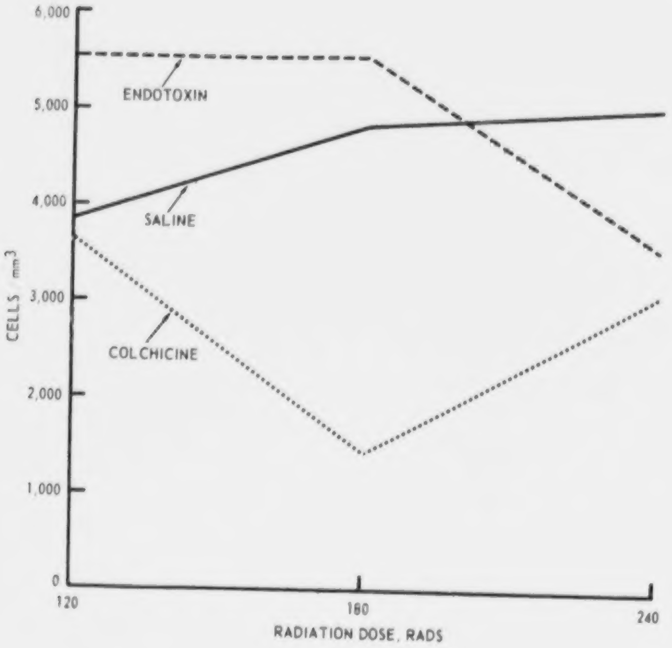

Figure 49. Effect of Radiation Dose and Drugs on Nucleated Red Blood Cell Counts.

E. HEMATOLOGICAL AND ERYTHROPOIETIC RESPONSE IN IRRADIATED FETAL RATS

Roger A. Budd, DeWitt G. Hazzard, Jesse Y. Harris

Fetal rats were exposed *in utero* at 15 days gestation to 300 rads X radiation. Erythropoietic and hematological changes were determined from 17 to 21 days gestation.

Radiation significantly altered body weights as a function of time after irradiation (Figure 50). Body weights for controls and irradiated fetuses increased exponentially between 17 and 21 days gestation. Body weight increases of irradiated fetuses appeared to be delayed, with irradiated animals achieving the approximate weight of the controls one day later in gestation.

Irradiated fetuses incorporated more ^{59}Fe than control fetuses (Figure 51). The incorporation of ^{59}Fe in both groups decreased linearly with time with no significant difference in the rate of decrease.

The hematocrits of irradiated and control fetuses generally increased with time (Figure 52). At 17 days gestation (2 days post-irradiation) the groups were similar; however, at 18 days gestation the hematocrits of irradiated fetuses were approximately 70 percent of the controls. Between 20 and 21 days gestation the hematocrits of the irradiated fetuses were again similar to the controls. These results suggest an initial impairment to the development or release of circulating erythroid tissue followed by subsequent hematological recovery.

Total leukocyte counts of the controls did not change appreciably from 17 to 21 days gestation. There was transient leukocytosis in the irradiated fetuses at 17 days gestation followed by marked leukopenia by 21 days gestation (Figure 53).

Alterations in body weights, hematocrits and in ^{59}Fe incorporation of irradiated fetal rats indicate a radiation induced developmental time delay of about one day. These data suggest an erythropoietic repair mechanism in fetal animals following damage by X irradiation.

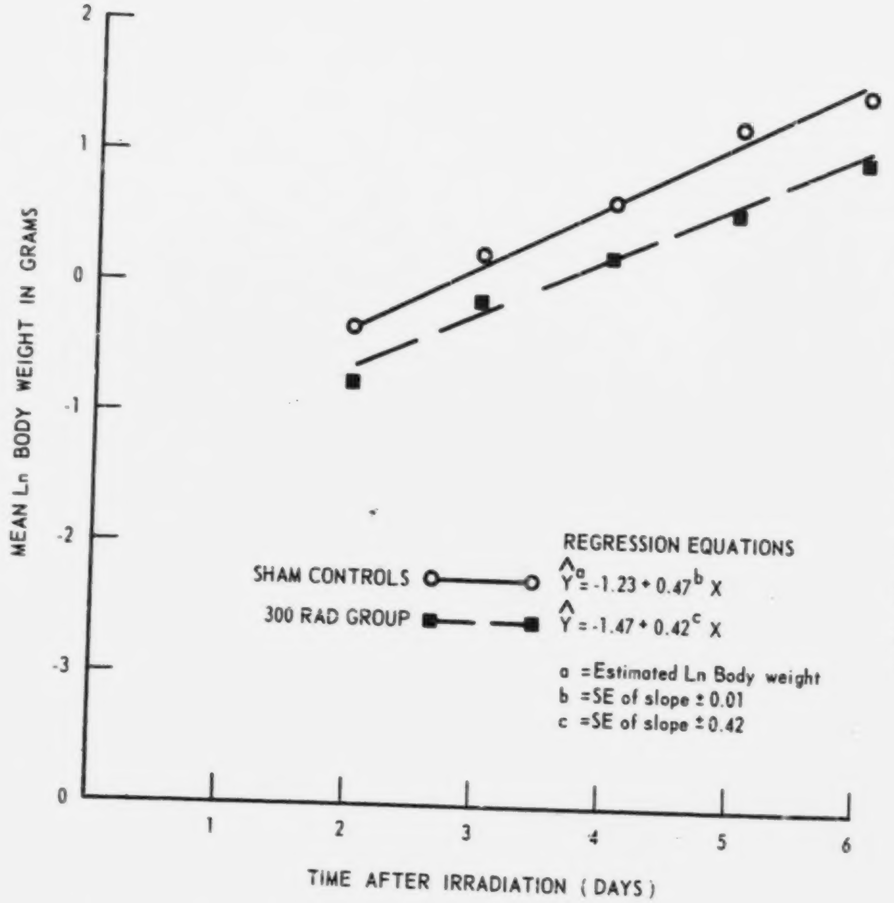

Figure 50. Body Weight Changes of Fetal Rats Following X Irradiation at 15 Days Gestation.

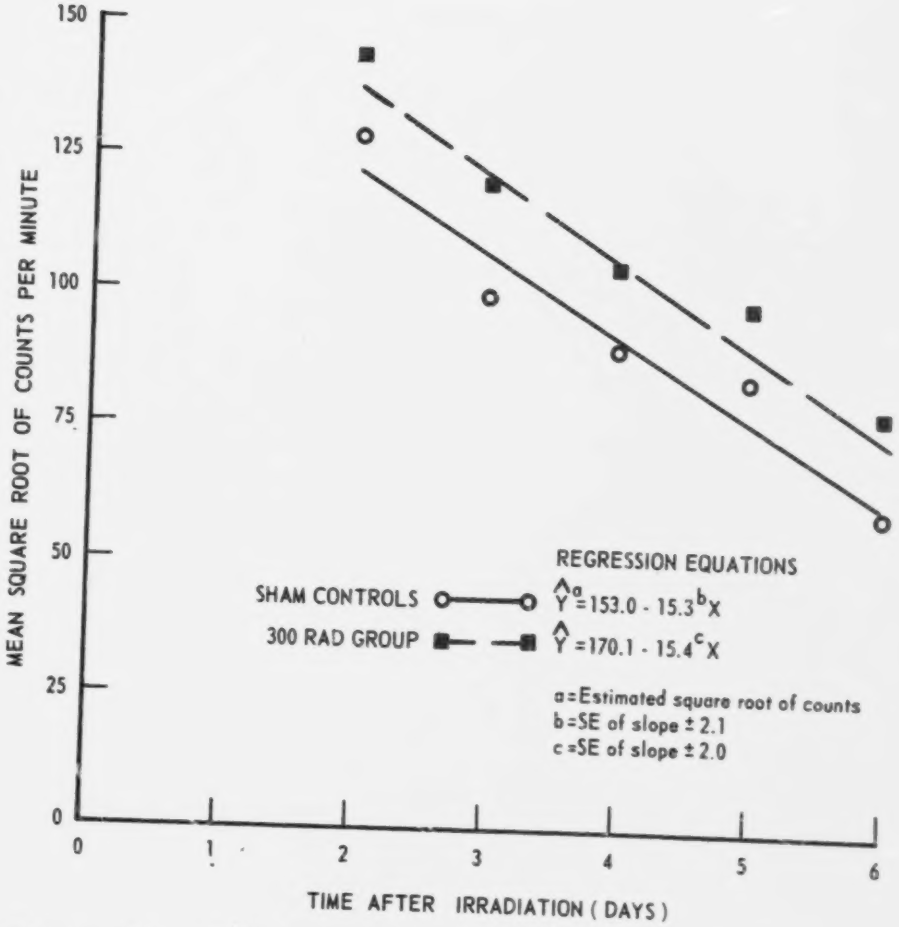

Figure 51. Incorporation of ^{59}Fe in Circulating Blood of Fetal Rats Following X Irradiation at 15 Days Gestation.

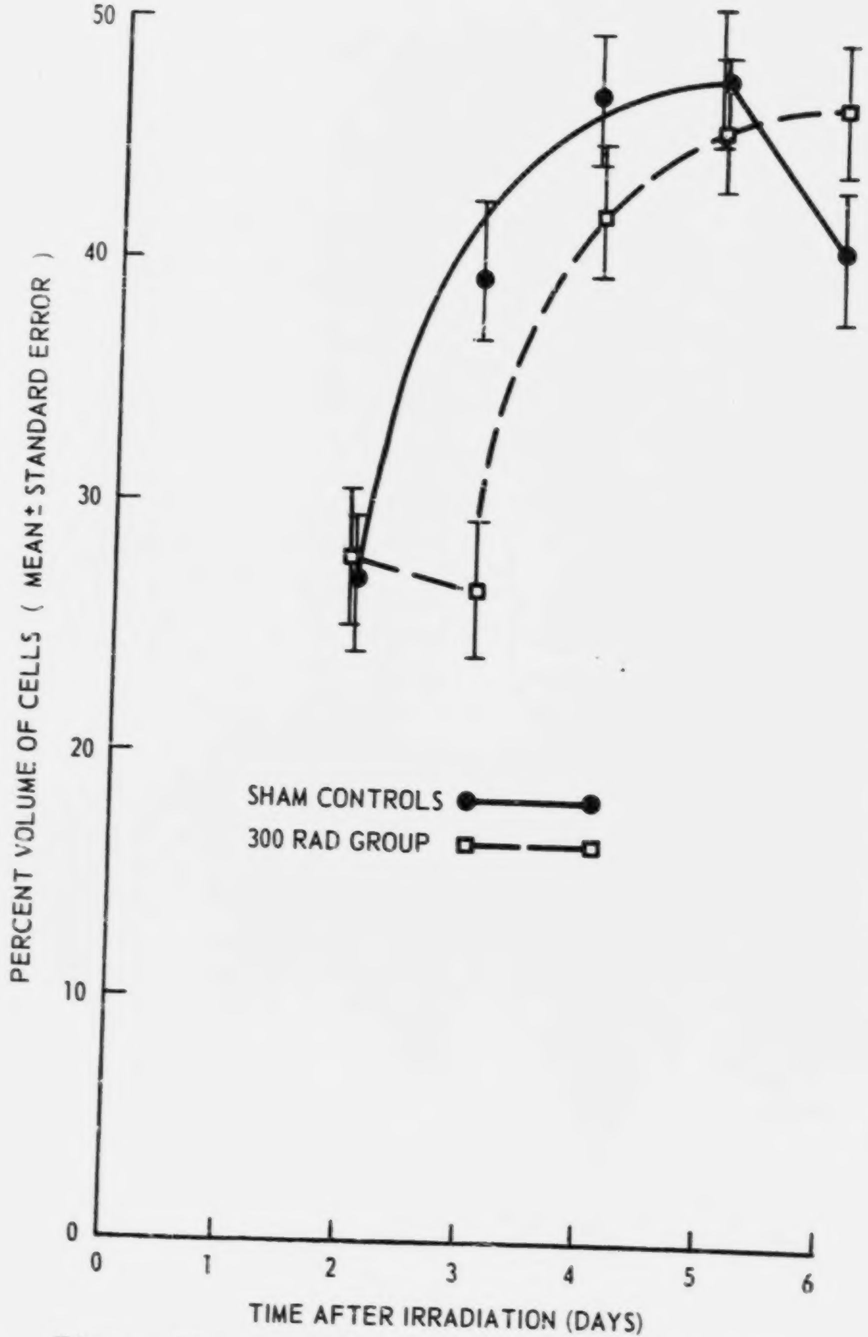

Figure 52. Hematocrit Changes of Fetal Rats Following X Irradiation at 15 Days Gestation.

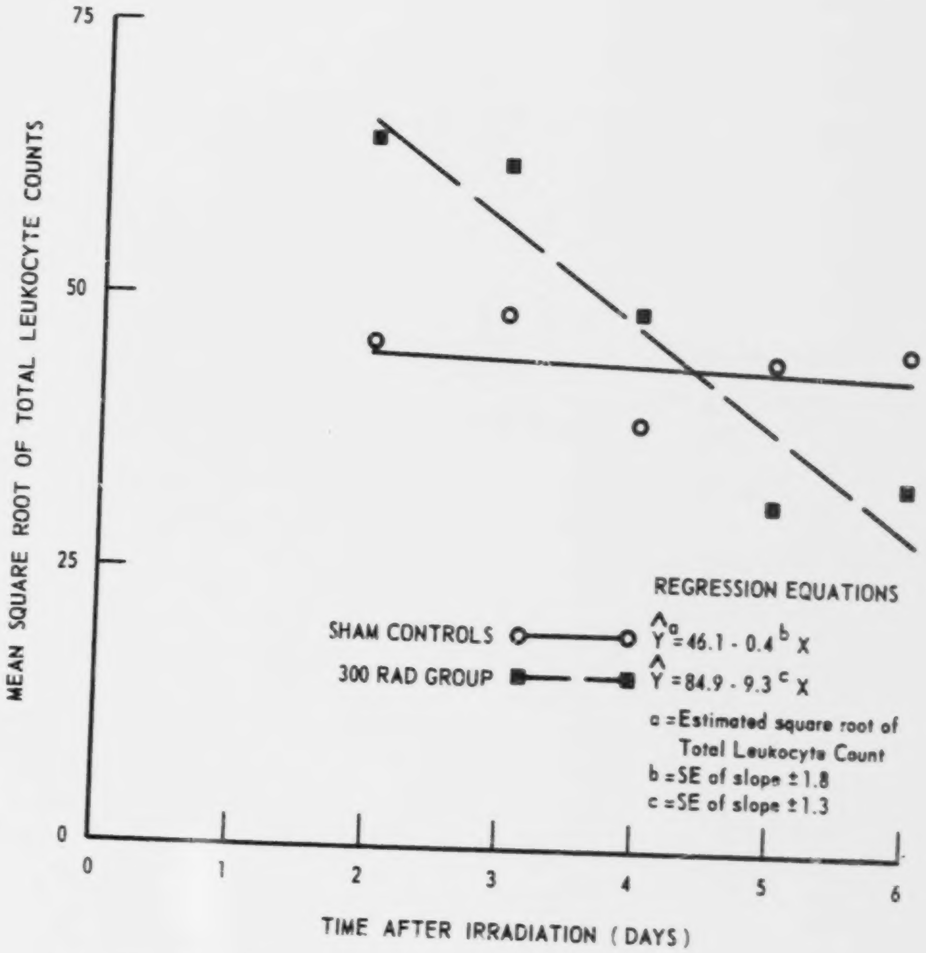

Figure 53. Total Leukocyte Counts Following X Irradiation of Fetal Rats at 15 Days Gestation.

F. EFFECT OF PRETREATMENT WITH ENDOTOXIN ON HEMATOLOGICAL AND ERYTHROPOIETIC RESPONSE IN IRRADIATED FETAL RATS

Roger A. Budd, DeWitt G. Hazzard, Jesse Y. Harris

Since the fetal hematological system is sensitive to ionizing radiation, experiments were carried out to determine if in utero pretreatment with endotoxin before irradiation favorably affected fetal hematological and erythropoietic tissues. The effects of endotoxin were evaluated at the 0, 175, and 350 rad radiation levels.

Fetal rats pretreated with endotoxin and exposed to 175 or 350 rads X radiation had higher leukocyte counts than saline-treated animals. At the higher radiation levels, endotoxin-treated fetuses gained more weight than saline-treated animals. Endotoxin treatment had no effect on ^{59}Fe incorporation or hematocrits. The results of this study are graphically presented in Figures 54-57.

The greater leukocyte counts may indicate that endotoxin received in utero can offer radioprotection to the fetal hematological system. However, the toxic effect of the material itself as evidenced by more than one-third of the fetuses in a state of resorption presently negates its possible use for this purpose.

G. COMPARATIVE RESPONSE TO 30 kVp AND 250 kVp X RAYS OF THE MOUSE HEMATOLOGICAL SYSTEM

Roger A. Budd, Richard P. Chiacchierini, DeWitt G. Hazzard, Jim Rolofson, Sarah Jane Smith

There is growing concern about the possible biological hazards of X rays that are emitted from color television receivers (30 kVp). Therefore, a comparative study between 30 kVp and 250 kVp X rays was undertaken to examine possible alterations in the mouse hematological system. Post-irradiation observations were made one day after whole-body mid-line absorbed doses of 0, 30, 60, 120, and 240 rads.

Both 30 kVp and 250 kVp X rays significantly decreased (all at $P < .01$) splenic uptakes of ^{131}I labeled iododeoxyuridine (^{131}IUdR) and ^{59}Fe, total leukocyte counts, ^{59}Fe incorporation into erythrocytes, and spleen weight.

When observations were averaged over all dose levels, there were no statistically significant differences in total leukocyte counts, ^{59}Fe incorporation into erythrocytes, and spleen weights between animals receiving 30 kVp or 250 kVp X irradiation. Animals irradiated with 30 kVp X rays had significantly lower ($P < .05$) splenic uptakes of ^{131}IUdR and ^{59}Fe compared to animals that received 250 kVp X rays. The results of this study are graphically presented in Figures 58-62.

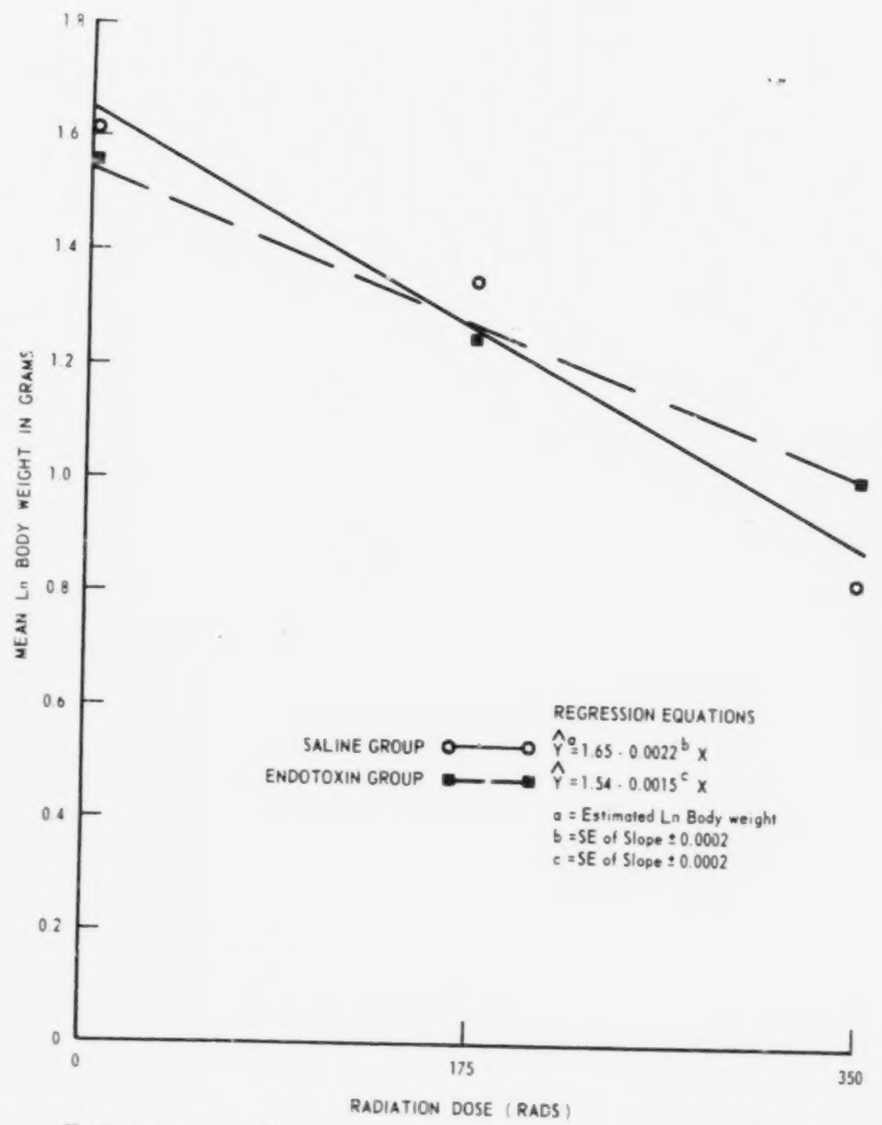

Figure 54. Relationship between Body Weight and Radiation in Fetal Rats Injected with Saline or Endotoxin.

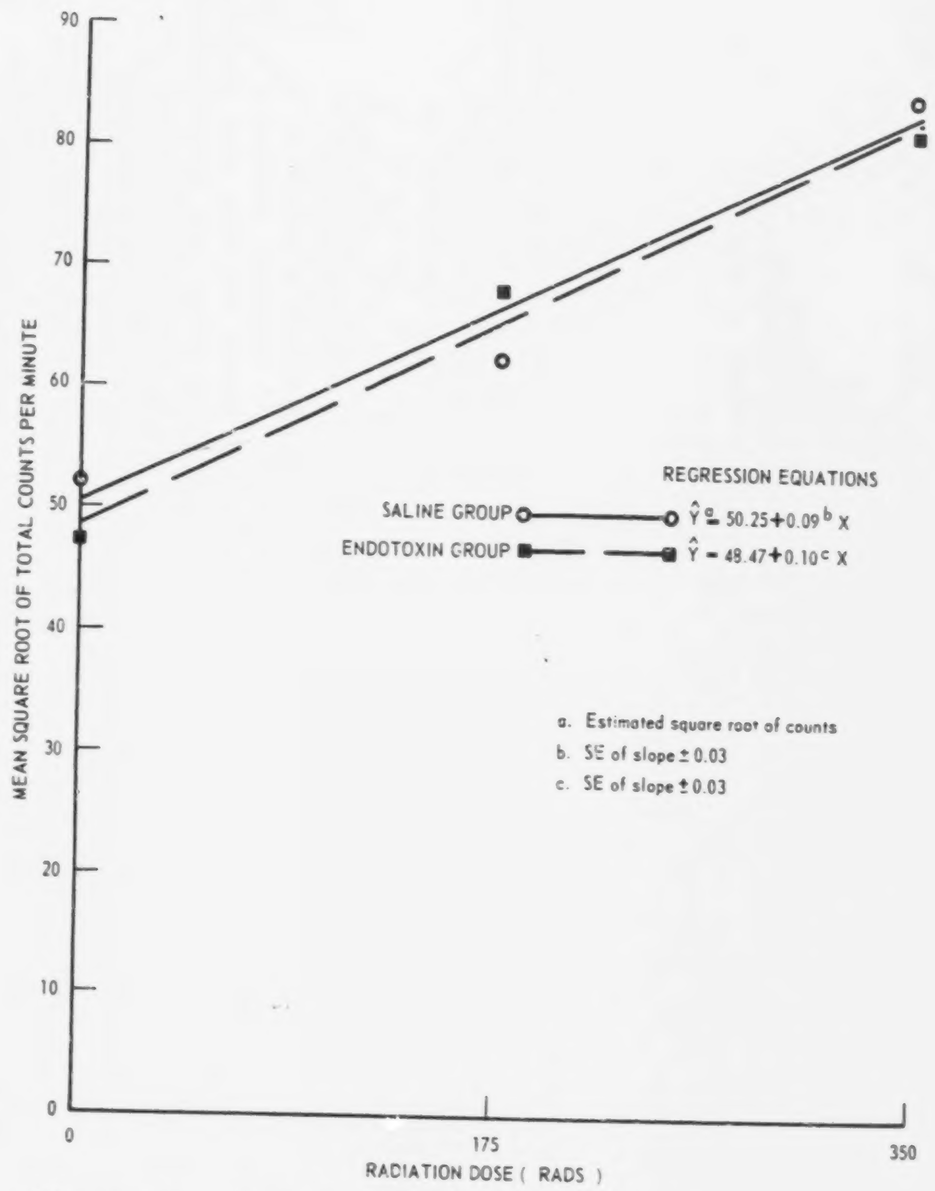

Figure 55. Relationship between ^{59}Fe Incorporation and Radiation Dose in Fetal Rats Injected with Saline or Endotoxin.

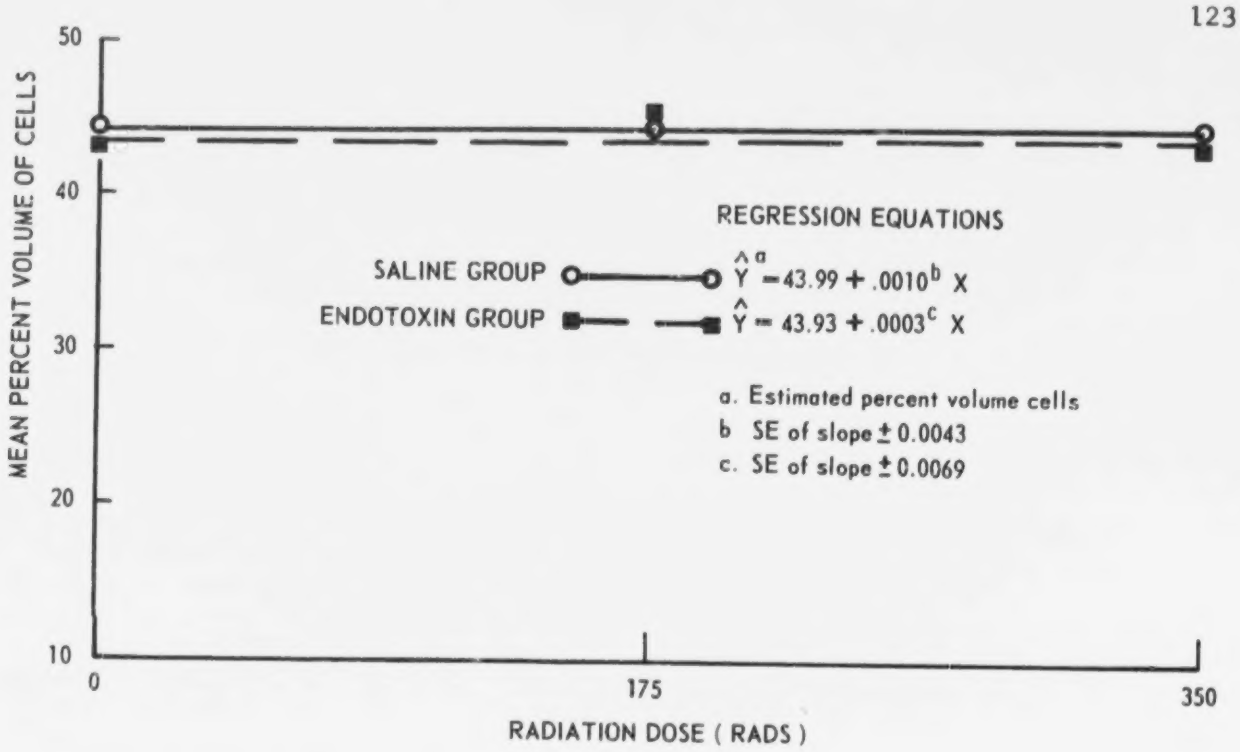

Figure 56. Relationship between Hematocrit Values and Radiation Dose in Fetal Rats Injected with Saline or Endotoxin.

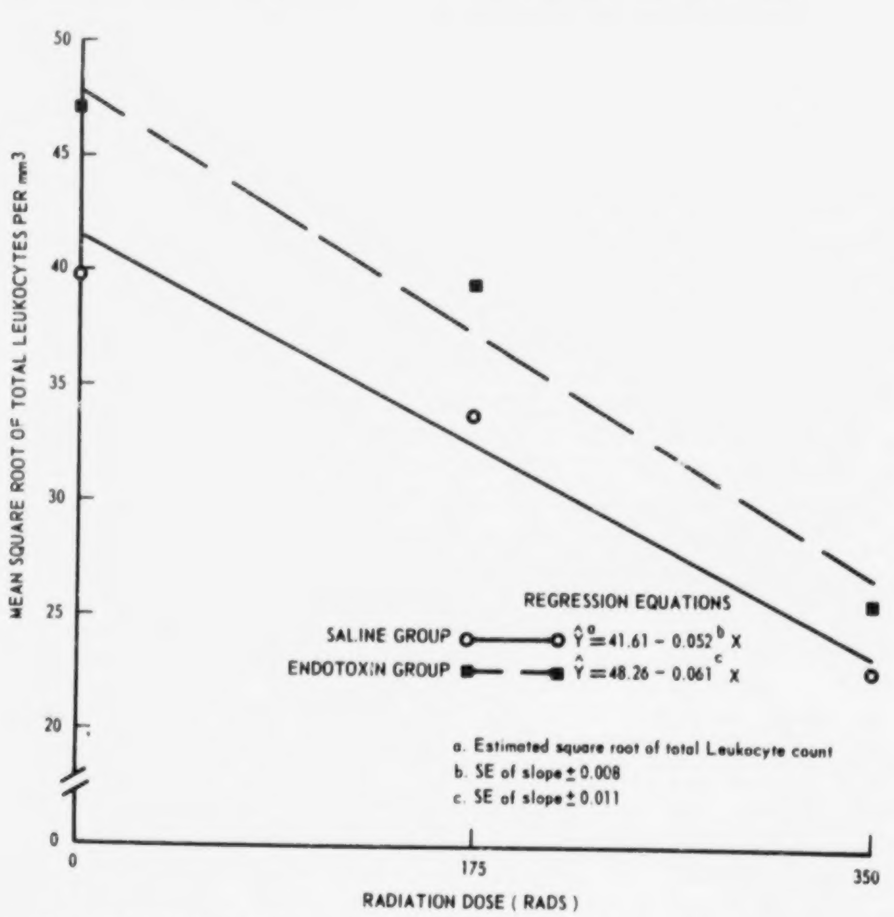

Figure 57. Relationship between Total Leukocyte Counts and Radiation Dose in Fetal Rats Injected with Saline or Endotoxin.

The results of this study suggest that there is little difference between the effects of 30 kVp and 250 kVp X rays on total leukocyte counts, ^{59}Fe incorporation into erythrocytes, spleen weights, body weights, and hematocrits when measured one day postirradiation. The lower splenic uptakes of ^{131}IUdr and ^{59}Fe in animals irradiated with 30 kVp X rays suggest a greater biological effect compared to 250 kVp X rays.

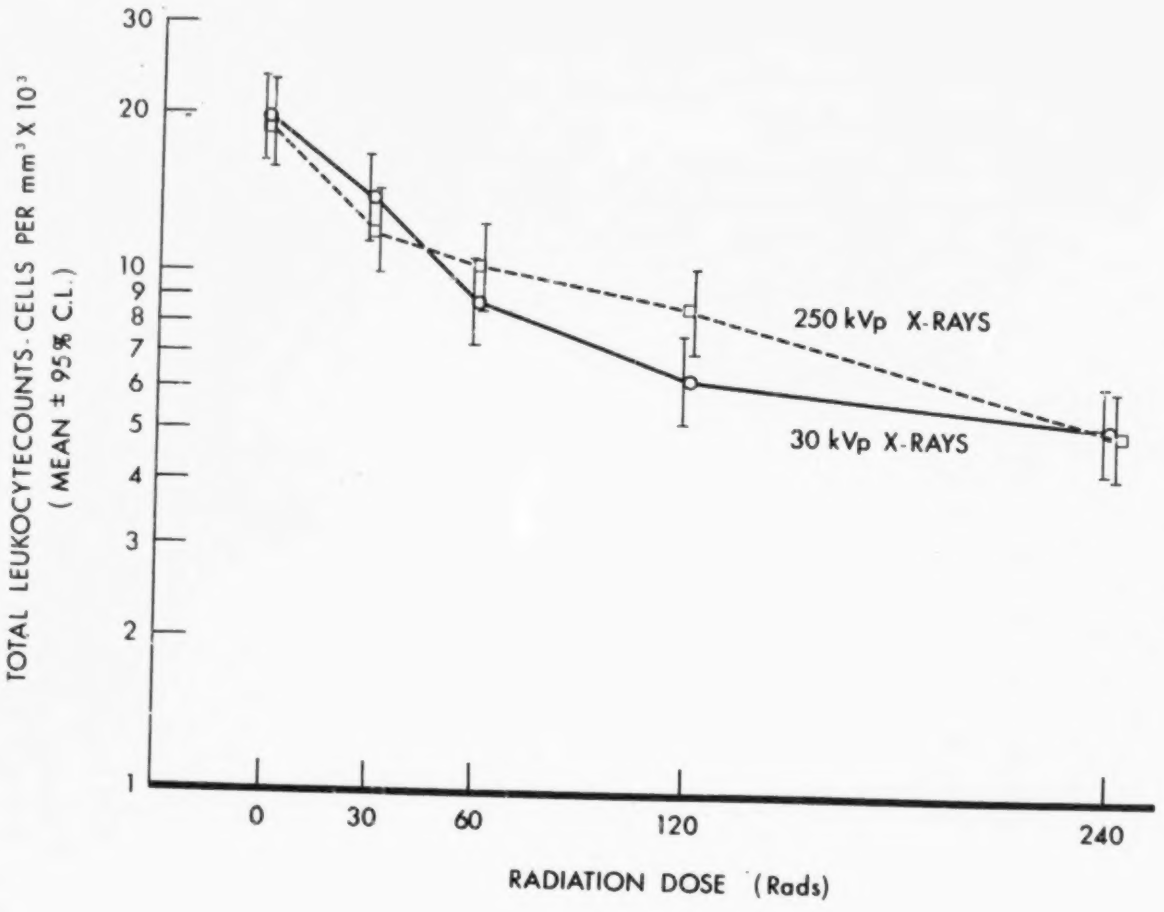

Figure 58. Total Leukocyte Count Response as a Function of Dose. Observations made 24 hours postirradiation.

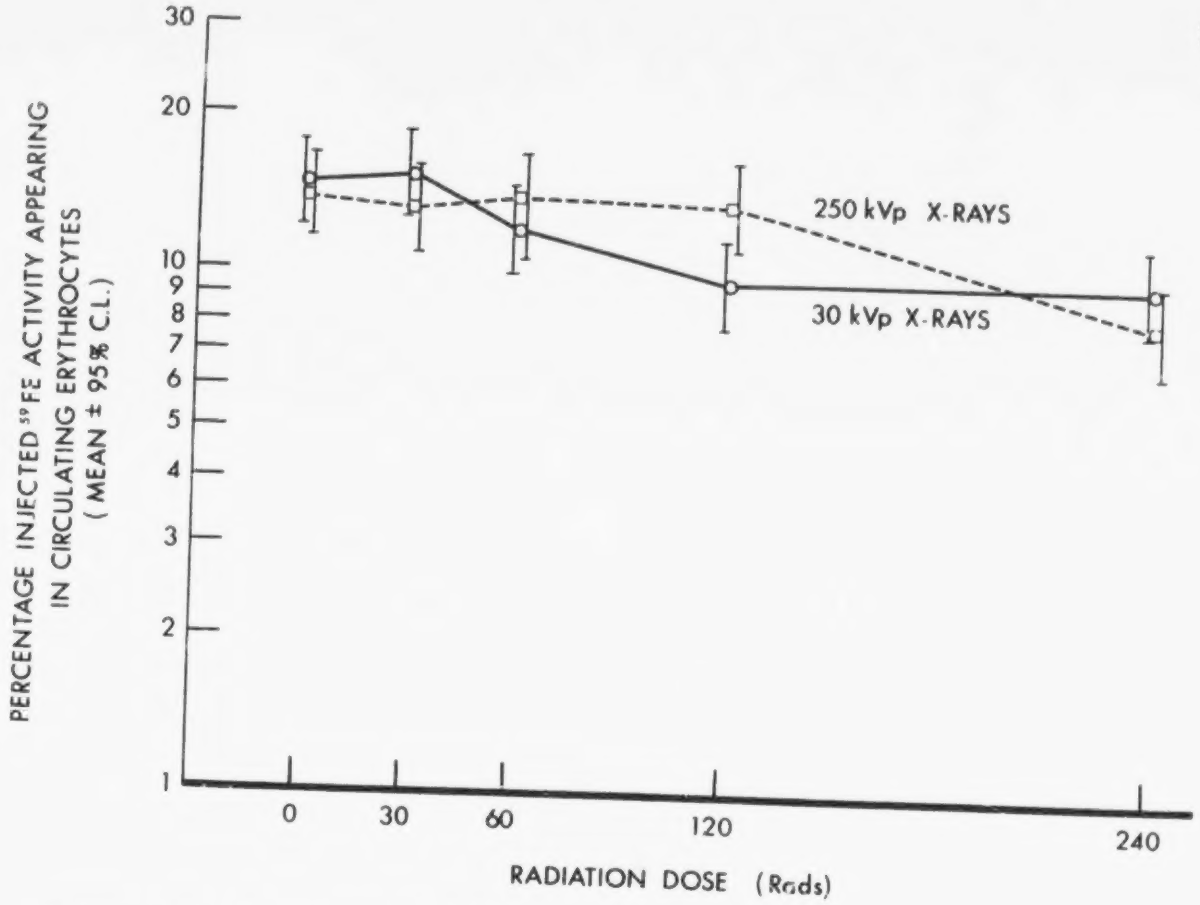

Figure 59. Response of ^{59}Fe Incorporation into Erythrocytes as a Function of Dose. Observations made 24 hours postirradiation.

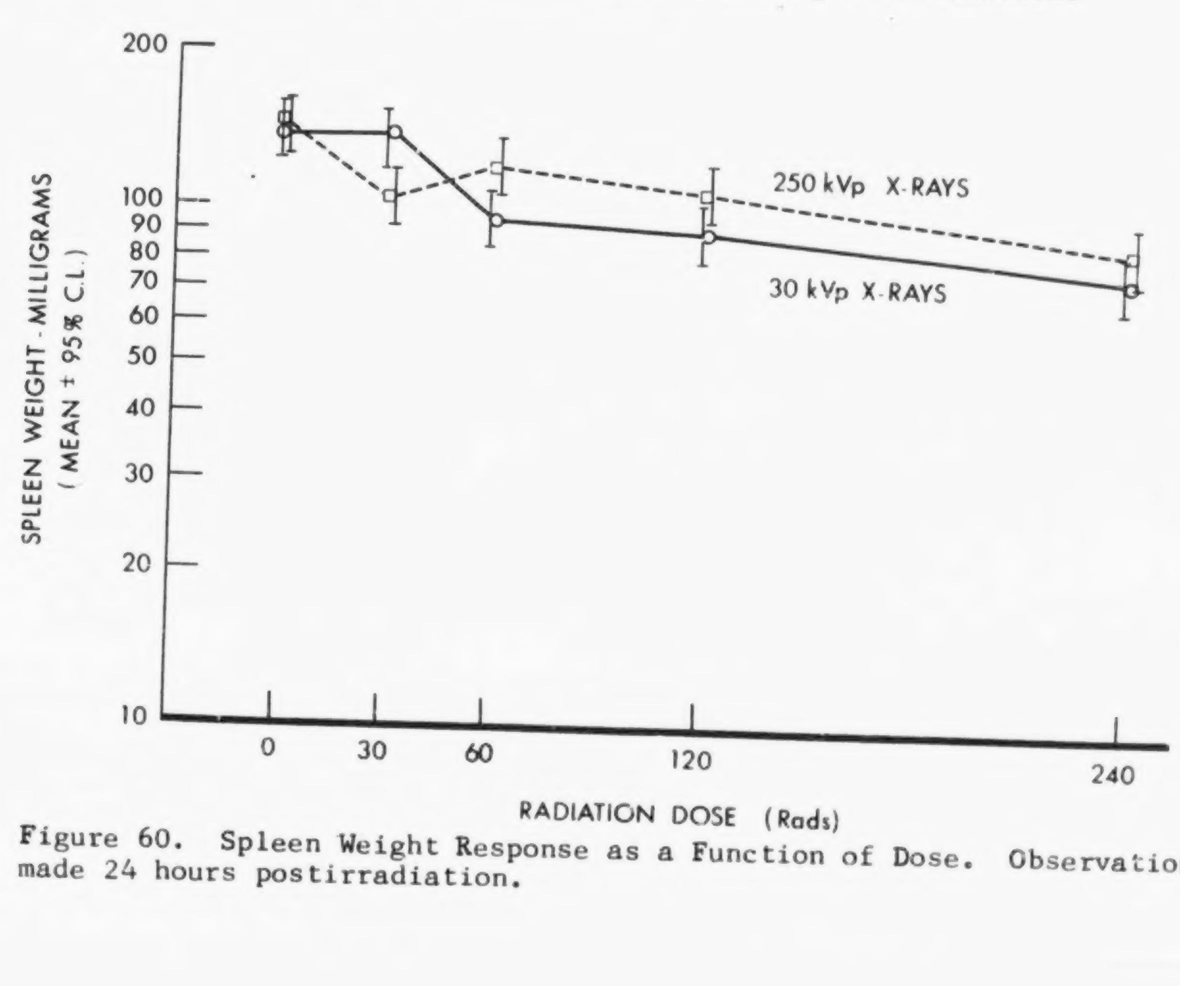

Figure 60. Spleen Weight Response as a Function of Dose. Observations made 24 hours postirradiation.

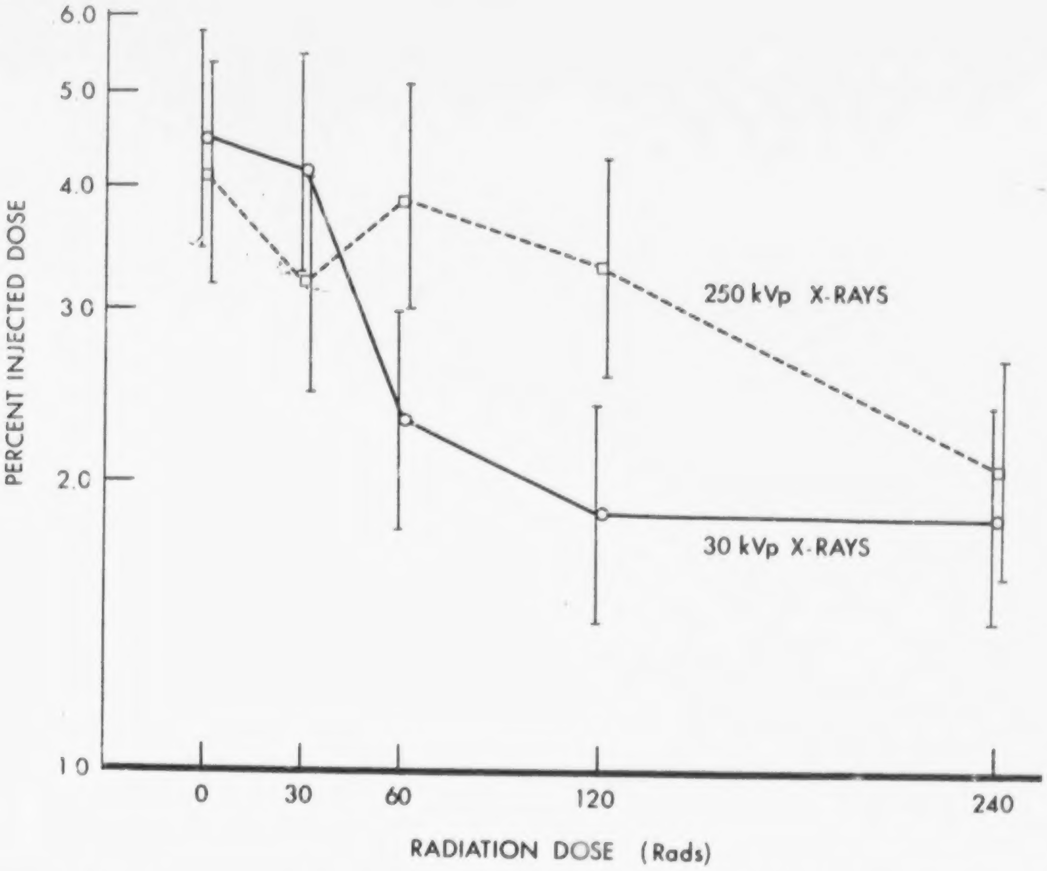

Figure 61. Response of Splenic Uptake of ^{59}Fe as a Function of Dose. Observations made 24 hours postirradiation (mean ± 95% C.L.).

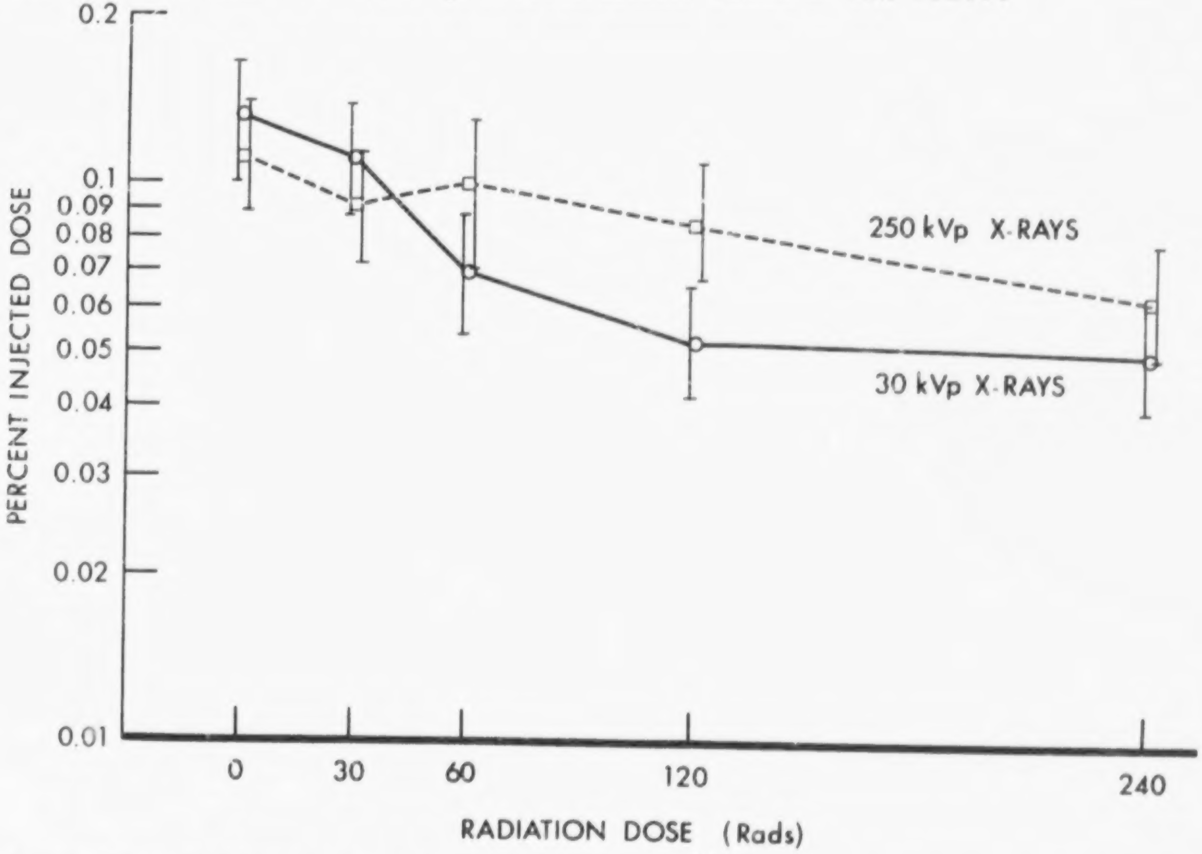

Figure 62. Response of Splenic Uptake of ^{131}IUdR as a Function of Dose. Observations made 24 hours postirradiation (mean ± 95% C.L.).

H. IMPAIRED INCORPORATION OF LABELED PHENYLALANINE INTO PROTEIN IN THE X-IRRADIATED FETAL RAT

Moris L. Shore, John W. Laskey, Lawrence R. Simmons, Gordon L. Jessup, Jr., and Katherine M. Selby

Introduction

Bar and Boullé first suggested the possibility that *in utero* exposure of the developing fetus to ionizing radiation could affect post partum survival.[1] Since that time numerous studies have been performed that have investigated biological damage produced by *in utero* radiation. These have been extensively reviewed.[2,3]

Periods exist during development when irradiation causes prenatal death, abnormalities, or neonatal death in the developing fetus. Prenatal death has generally been associated with irradiation during the preimplantation period; abnormalities and neonatal death, with irradiation during the period of major organogenesis.

A number of investigators have suggested that the deleterious effects of *in utero* irradiation may result from cell killing and mitotic delay. However, it has also been demonstrated that irradiation during the preimplantation period increases the subsequent incidence of cataracts [4] and exencephaly.[5] The studies that have been performed indicate that ionizing radiation can have a serious impact on embryological development. It is clear that certain aspects of the radiation effect may be a consequence of mortality at the cellular level. It is equally apparent that cells which survive irradiation may be altered and may have impaired developmental or functional capacity.

Work in this laboratory has demonstrated that a decreased capacity to synthesize liver enzymes exists in the rat at birth, following irradiation in utero.[6] Similar observations have been made in the dog.[7] It has further been demonstrated that irradiation inhibits the normal increase in enzyme activity that occurs in the developing rat brain.[8]

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[1] Bar, M.M.P, and T. Boullé. Bull. Soc. Obstet. Gynec. (Paris) 4:251, 1901.

[2] Russell, L.B. The effects of radiation on mammalian prenatal development. *In* Radiation Biology, Vol. I, p. 861-918, A. Hollaender (ed.), McGraw-Hill Book Co., Inc. New York, 1954.

[3] Jacobsen, L. Low dose X-irradiation and teratogenesis, Acta Pathol. Microbiol. Scandinavica, Suppl. 193: Supp. No. 1, 1968.

[4] Rugh, R. et al. Biol. Bull 131:145-154, 1966.

[5] Rugh, R. and E. Grupp. J. Neuropathol. Exptl. Neurol. 18:468-481, 1959.

In the studies cited above, the observation of altered protein synthesis was made a number of days after irradiation. Thus, it is possible that indirect factors such as nutritional state of the animal after irradiation may be involved in the observed alterations of protein synthesis.

The present study investigates the effect of fetal irradiation on growth and the fetal incorporation of labeled phenylalanine into protein 24 hours after irradiation. The results of this study indicate that radiation caused a decrease in growth and labeled amino acid incorporation in the developing embryo.

Method

Six days pregnant Sprague-Dawley rats were obtained from a commercial source. On day 13 of gestation (day of conception = day zero), pregnant rats were either sham-irradiated or exposed to 250 kVcp X rays. X-irradiated animals received a total dose of 180 rads at a dose rate of 83 rads per minute. Incorporation of ^{14}C-labeled phenylalanine into protein was measured 24 hours post-irradiation. In vivo incorporation of labeled amino acid into fetal protein was measured 20 minutes after the intraperitoneal administration of ^{14}C phenylalanine. In vitro incorporation was measured in a fetal post-mitochondrial cell free system previously described.[9]

Results and Discussion

Table 18 presents data on the body weights of normal fetuses on day 13 of gestation and sham- and X-irradiated fetuses on day 14 of gestation, from either fed or starved pregnant rats. Fetuses in all experimental groups increased in weight significantly between day 13 and day 14 of gestation. However, the rise in body weight seen in both X-irradiated groups was significantly less than that seen in the respective controls ($P<0.05$). No significant difference in weight was observed between X-irradiated fetuses from either fed or starved rats. Similarly, no significant difference in weight was observed in sham-irradiated fetuses from either fed or starved rats. It would thus appear that stunting was produced by exposure of the animals to ionizing radiation, and was unrelated to maternal food and water intake during the 24-hour period following exposure to radiation.

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[6] Smith, C.H. and M.L. Shore. Radiation Res. 29:499-504, 1966.

[7] Wagner, M.T., Jr. Influence of Gamma Irradiation In Utero on the Development and Functional Adaptation of Selected Enzymes in the Beagle. Doctoral Dissertation, Colorado State University, 1967.

[8] de Vellis, J. et al. J. Neurochem. 14:499-511, 1967.

[9] Shore, M.L., J.W. Laskey, L.R. Simmons, and G.L. Jessup, Jr. Ninth Annual Hanford Biology Symposium, 1969.

TABLE 18. BODY WEIGHTS OF FETUSES ON DAY 14 OF GESTATION FROM FED OR STARVED, SHAM- OR X-IRRADIATED RATS

	Fetal Body Weight (mg)		
Treatment	Fed	Starved	
Shammed	145 ± 4 (10)	139 ± 4 (11)	$P < .05$
X-Irradiated	121 ± 3 (13)	117 ± 4 (11)	$P < .05$
	$P < .05$	$P < .05$	

Fetal weight on day 13 of gestation was 52.4 ± 2.0 mg.
Values are means of mean litter weights ± S.E. numbers in parentheses indicate no. of litters examined.
Pregnant animals were sham- or X-irradiated on day 13 of gestation.

The data on the relative incorporation of ^{14}C-Phe into the acid insoluble fraction of fetuses from fed sham- and X-irradiated rats suggest varible amounts of the intraperitoneally administered ^{14}C-Phe may have been available to fetuses for incorporation into protein. Thus, the amount of available ^{14}C-Phe was approximated by the sum of ^{14}C-Phe A.I.F. and ^{14}C-Phe A.S.F. This is a minimum value since it neglects loss of ^{14}C-Phe A.I.F. as a result of protein degradation. Relative incorporation was expressed as ^{14}C-Phe A.I.F./(^{14}C-Phe A.S.F. + ^{14}C-Phe A.I.F.). Although a trend toward lower values was suggested in the irradiated group, the difference was not significant ($P < 0.05$). Since variable food and water consumption by experimental animals may have masked a radiation effect, the relative incorporation of ^{14}C-Phe into the A.I.F. was also measured in fetuses from pregnant animals that were starved following sham- or X-irradiation.

The relative incorporation of ^{14}C-Phe into the A.I.F. in the irradiated group was less than that observed in the shammed group ($P < 0.05$). An overall analysis of variance of the data in Table 19 showed statistically significant effects due to both X radiation ($P < 0.05$) and food and water deprivation ($P < 0.05$). No indication of interaction between these two factors was detected, thus the effects are additive.

TABLE 19. RELATIVE INCORPORATION, IN VIVO, OF ^{14}C · PHE INTO THE A.I.F. OF FETUSES FROM FED OR STARVED, SHAM- OR X-IRRADIATED RATS

	Relative Incorporation (^{14}C · Phe A.I.F./^{14}C · Phe A.S.F. + ^{14}C · Phe A.I.F.)		
Treatment	Fed	Starved	
Shammed	0.394 ± 0.011 (7)	0.354 ± 0.010 (8)	$P < .05$
X-Irradiated	0.370 ± 0.010 (9)	0.322 ± 0.010 (8)	$P < .05$
	$P < .05$	$P < .05$	

Incorporation values were obtained 24 hours after sham- or X-irradiation and are measured in the fetus per unit weight of fetal tissue. Values are means ± standard error of the mean determined from the pooled variance obtained in the analysis of variance.
Numbers in parentheses indicate no. of litters examined.

The multiple range test[10] indicated that incorporation in starved-shammed and starved-irradiated groups was significantly less than incorporation in the respective fed groups ($P < 0.05$). Further, it indicated that ^{14}C-Phe incorporation in the starved irradiated group was significantly lower than the incorporation observed in any of the other experimental groups ($P < 0.05$). Thus these data indicate that, in the fetus present in its intra-uterine environment, X-irradiation causes a small but significant decrease in the incorporation of ^{14}C-Phe into protein per unit weight of fetal tissue.

Irradiated fetuses increased in weight from day 13 to day 14 to the extent of only 72 percent of the increase observed in the unirradiated fetuses. Thus, expressed in terms of relative incorporation per whole fetus, the differences were greater than the differences in relative incorporation per unit weight of fetal tissue.

Table 20 also presents data on the incorporation of ^{14}C-Phe into the A.I.F. in fetal cell-free systems. Since precursor ^{14}C-Phe was maintained constant in the cell-free systems employed in these studies, incorporation is expressed as ^{14}C-Phe incorporated into the A.I.F.

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[10] Duncan, D.B. Biometrics 11:1-42, 1955.

Incorporation of ^{14}C-Phe into the A.I.F. in the fetal cell-free system of both the fed group and the starved group was reduced by irradiation ($P < 0.05$). Thus, the data indicate that, in all cases, fetal incorporation of ^{14}C-Phe into the A.I.F., as measured by the cell-free system, was reduced by fetal exposure to X rays. Incorporation in shammed animals was significantly reduced by food and water deprivation ($P < 0.05$). However, incorporation in irradiated animals was not affected by food and water deprivation. It was not determined whether fetuses derived from starved irradiated animals differed nutritionally from fetuses derived from fed irradiated animals. Thus the results which have been obtained indicate that while maternal food and water deprivation decreases incorporation of ^{14}C-Phe in the fetus, irradiation causes a still further decrease in the incorporation of ^{14}C-Phe into fetal protein.

The irradiation was accompanied not only by a depression in in vitro incorporation but also by a decreased fetal growth rate. Thus expressed in terms of incorporation per whole fetus (incorporation per gram x fetal weight) the differences were greater than those based on incorporation per unit weight of fetal tissue.

It should be noted that the data presented in this study measure incorporation of a labeled precursor into protein. An effort has been made to estimate (in vivo studies) or control (in vitro studies) the amount of label available for incorporation. However, no information was obtained on precursor pool size and precursor specific activity. Thus, it is not possible to equate decreased incorporation of amino acid into acid insoluble fraction with decreased protein synthesis. The decreased growth of the irradiated fetuses suggests that the total pool of protein in the irradiated fetuses did not increase to the same degree as it did in the sham-irradiated fetuses during the 24-hour period following irradiation. Such a finding would be consistent with either decreased synthesis of protein or an increased rate of degradation of protein with no change in the normal pattern of protein synthesis as a consequence of irradiation.

The difference between incorporation in sham- and X-irradiated fetuses was small though significant in the in vivo studies with starved animals. In the studies with the cell-free system derived from sham- and X-irradiated fetuses the differences were much greater. The latter studies examined incorporation in the post-mitochondrial supernatant and thus measure cytoplasmic incorporation to the exclusion of mitochondrial and nuclear incorporation of labeled precursor into the A.I.F. Studies currently in progress are investigating the effect of fetal irradiation on cytoplasmic, nuclear, and mitochondrial protein synthesis.

Conclusions

The present study has investigated the effect of fetal irradiation on fetal growth and fetal incorporation of labeled phenylalanine into the acid insoluble fraction of the fetus, both in vivo and in a fetal derived cell-free system. The following observations were made relative to the 24-hour period following fetal irradiation:

1) The increase in fetal weight observed in the irradiated fetuses was significantly less than that observed in unirradiated fetuses.

2) The in vivo incorporation of labeled phenylalanine into the acid insoluble fraction of the fetus shows a small but significant decrease following irradiation.

3) The incorporation of labeled phenylalanine into the acid insoluble fraction of a fetal derived cell-free system was reduced following irradiation, to a greater extent than that observed in the in vivo studies.

TABLE 20. INCORPORATION OF ^{14}C · PHE INTO THE A.I.F. OF A CELL-FREE SYSTEM FROM FETUSES OBTAINED FROM FED OR STARVED, SHAM- OR X-IRRADIATED RATS

	Incorporation (dpm/ g fetus) x 10^{-3}		
Treatment	Fed	Starved	
Shammed	35.7 ± 2.1 (5)	28.0 ± 2.1 (5)	$P < .05$
X-Irradiated	23.5 ± 2.1 (5)	20.5 ± 2.1 (5)	$P < .05$
	$P < .05$	$P < .05$	

Incorporation values were obtained 24 hours after sham- or X-irradiation, and are measured in an amount of post mitochondrial supernatant associated with a unit weight of fetal tissue.
Values are means ± standard error of the mean determined from the pooled variance obtained in the analysis of variance.
Numbers in parentheses indicate no. of litters examined.

I. PERSISTENCE OF DEPRESSED INCORPORATION OF LABELED PHENYLALANINE INTO FETAL RAT PROTEIN AFTER *IN UTERO* X IRRADIATION

John Laskey, Lawrence Simmons, Katherine Selby, Moris Shore

The data reported in Section H indicate that a decrease in growth and labeled amino acid incorporation in the developing embryo is observed 24 hours following irradiation. It was important to determine whether the observed effect was transient or whether it persisted beyond the 24-hour period following exposure, particularly in view of of our previous work demonstrating impaired enzyme synthesis at birth following exposure *in utero*. The present study extends our observations of in vitro labeled amino acid incorporation into fetal protein to the period of 48 and 72 hours after exposure to ionizing radiation.

Sprague-Dawley rats were irradiated in utero (180 rads, 250 kVcp X rays delivered to pregnant mothers on day 13 of gestation). Fetal weight and incorporation of labeled amino acid into protein were measured 24, 48, and 72 hours following irradiation. Since the observations cited above were made a number of days following irradiation, alterations in the nutritional state were investigated in conjunction with radiation exposure.

Materials and Methods

Eight days pregnant Sprague-Dawley rats were obtained from a commercial source. On day 13 of gestation (day of conception = day zero), pregnant rats were either sham-irradiated or exposed to 250 kVcp X rays. The sham- and X-irradiation were performed simultaneously. X-irradiated animals received a total dose of 180 rads at a dose rate of 83 rads per minute. Animals in lucite containers were placed 50 cm from the X-ray source which was filtered with 0.5 mm copper and 1.0 mm aluminum. The half value layer was 0.53 mm in copper.

Subsequent to sham- or X-irradiation, the pregnant rats were either allowed food and water until sacrifice (fed), or deprived of food and water for 24 hours prior to sacrifice (starved). Twenty-four, 48, or 72 hours following sham- or X-irradiation, pregnant rats were sacrificed by decapitation and the fetuses obtained for the determination of in vitro incorporation of ^{14}C-phenylalanine (^{14}C-Phe) into the acid insoluble fraction (AIF) of a fetal post-mitochondrial supernatant (PMS) cell-free system.

When pregnant animals were sacrificed, the fetuses of a single litter were removed, weighed, and put into a single Dounce homogenizer, in 1.7 volumes of Tris-HCl buffer (.1 M Tris, 0.005 M $MgCl_2$, 0.15 M sucrose, 0.0025 M KCl and 0.005 M mercaptoethanol with the pH adjusted to 7.3 with HCl). The fetuses were homogenized with ten strokes in the Dounce homogenizer, and the homogenates were then centrifuged at 15,000 G for 15 minutes to obtain a PMS. In vitro incorporation of ^{14}C-phenylalanine was measured in a fetal PMS cell-free system described in detail in the report which follows (p.137). This cell-free system differs in pH from the system used in the report immediately preceding, and increases the observed incorporation of ^{14}C amino acid into protein.

The components of the fetal PMS cell-free system were combined and maintained at 0° C prior to incubation. Incorporation was measured at 37° C for 30 minutes. Incorporation of labeled amino acid into protein was determined by the addition of 4 ml of 10 percent TCA to the incubation mixture. After 20 minutes at 0° C the precipitated incubation mixture was centrifuged and the pellets washed twice with 4 ml of 10 percent TCA. The second wash was heated in a 90° C water bath for 20 minutes and cooled to 0° C for 10 minutes prior to centrifugation. The pellets were then washed with alcohol:ether (3:1 by vol.), anhydrous ether, and air dried. The dried pellets were dissolved in 1 ml of concentrated formic acid and aliquots were counted in Bray's scintillant using a liquid scintillation spectrometer. Corrections for variable counting efficiency were made. Incorporation of ^{14}C-Phe into the A.I.F. is expressed as dpm per fetus. However, it should be noted that only the post-mitochondrial supernatant was used for the cell-free system. Thus, incorporation is measured in an amount of post-mitochondrial supernatant associated with a unit weight of fetal tissue.

Results and Discussion

Table 21 presents data on the body weights of normal fetuses on day 13 of gestation and of sham- and X-irradiated fed and starved fetuses on day 14, day 15, and day 16 of gestation (24, 48, and 72 hours post exposure, respectively). Fetuses in all experimental groups showed significant daily increases in weight between day 13 and day 16 of gestation. The fetal body weight in the irradiated groups was significantly less than that seen in the respective sham irradiated control groups ($P < 0.05$). Food and water deprivation of pregnant rats for 24 hours prior to sacrifice did not have a significant effect on the weight of the developing fetuses. It would thus appear that the stunting in animals exposed to ionizing radiation was unrelated to maternal food and water intake during the 72-hour period following exposure to radiation.

Table 22 presents the data on the incorporation of ^{14}C-phenylalanine into the acid insoluble fraction in the fetal post-mitochondrial supernatant cell-free system. Incorporation of ^{14}C-Phe into the acid insoluble fraction in fetuses of both fed and starved groups 24, 48, and 72 hours after exposure was depressed by irradiation ($P < 0.05$). In agreement with our observation in the preceding report, these data show that food and water deprivation in sham-irradiated animals reduces incorporation of ^{14}C-Phe in the fetal rat. However, radiation causes a still further and significant decrease in the incorporation of ^{14}C-Phe into fetal protein. The results which have been obtained in this study thus show that the decreased fetal incorporation of ^{14}C-Phe into the AIF, which is observed 24 hours after X irradiation, persists 48 and 72 hours after exposure to ionizing radiation.

TABLE 21. MEAN WEIGHTS OF RAT FETUSES FROM FED OR STARVED[a] SHAM-IRRADIATED OR X-IRRADIATED PREGNANT RATS

Hours Post Exposure[b]	Fed				Starved			
	Shammed		Irradiated		Shammed		Irradiated	
	mg	S.E.	mg	S.E.	mg	S.E.	mg	S.E.
0[c]	52.4	± 2 (10)[d]	--		--		--	
24[c]	145	± 4 (12)	121	± 3 (15)	139	± 4 (13)	117	± 4 (13)
48	238	± 7 (7)	192	± 8 (9)	251	± 7 (8)	188	± 5 (7)
72	403	± 13 (10)	325	± 13 (12)	413	± 10 (11)	297	± 8 (12)

a. Animals in starved group were without food and water for 24 hours prior to sacrifice.

b. All animals were irradiated on day 13 of gestation. Day of conception = day 0.

c. Data in these two rows were reported in an earlier study.

d. Numbers in parentheses = number of litters examined.

TABLE 22. INCORPORATION OF ^{14}C-PHENYLALANINE INTO THE ACID INSOLUBLE FRACTION OF A FETAL CELL-FREE SYSTEM OBTAINED FROM FED AND STARVED[a] SHAM-IRRADIATED AND X-IRRADIATED PREGNANT RATS

Hours Post Exposure[b]	Fed				Starved			
	Shammed		Irradiated		Shammed		Irradiated	
	dpm/g Fetus x 10	S.E.	dpm/g Fetus x 10	S.E.	dpm/g Fetus x 10	S.E.	dpm/g Fetus x 10	S.E.
24	55.7	±4.2 (4)[c]	37.0	±2.8 (6)	52.5	±2.4 (6)	35.2	±1.5 (6)
48	88.1	±3.3 (7)	55.1	±4.1 (8)	66.9	±3.0 (7)	48.0	±1.5 (7)
72	77.5	±4.5 (6)	59.8	±5.0 (6)	60.7	±0.8 (5)	50.0	±3.7 (6)

a. Animals in the starved group were without food and water for 24 hours prior to sacrifice.

b. All animals were irradiated on day 13 of gestation. Day of conception = day 0.

c. Numbers in parentheses = number of litters examined.

Summary

This study has investigatated the persistence of the radiation induced depression in fetal weight and the decrease in fetal incorporation of ^{14}C-labeled phenylalanine in a fetal post mitchondrial supernatant cell-free system. The following observations were made 24, 48, and 72 hours following in utero irradiation on day 13 of gestation:

1) With the exception of the observation 72 hours after exposure in fed sham- and X-irradiated animals, weight of X-irradiated fetuses was significantly less than that observed in the sham irradiated groups in both fed and starved animals during the 72-hour period following exposure.

2) The incorporation of ^{14}C-Phe into the acid insoluble fraction measured in the fetal cell-free system was reduced following X-irradiation. This observation was made 24, 48, and 72 hours following in utero exposure of fetal rats on day 13 of gestation.

J. PRECURSOR AMINO ACID POOLS, LABELED AMINO ACID INCORPORATION INTO PROTEIN AND PROTEIN SYNTHESIS IN IRRADIATED FETAL RATS

Lawrence Simmons, John Laskey, Moris Shore

In previous studies we have reported that fetal exposure to X irradiation is associated with depressed incorporation of labeled phenylalanine into protein. This depressed incorporation was observed in vivo 24 hours after irradiation (on day 13 of gestation), and in vitro, using a fetal post-mitochondrial supernatant (PMS) cell-free system 24, 48, and 72 hours following exposure to X rays.

No measurement of the precursor amino acid pool or precursor amino acid specific activity was made in the preceding studies. In the absence of this information no definitive conclusions could be drawn relative to the significance of the decreased incorporation of labeled amino acid which was observed after irradiation. Such decreased incorporation, for example, could have been the result of impaired synthesis of protein following irradiation. The decreased incorporation of labeled amino acid could have also resulted from an increase in the pool of phenylalanine in the irradiated animals, with a consequent decrease in the specific activity of the precursor amino acid, and no change in the actual amount of protein synthesis.

The present study utilizes a stable phenylalanine (^{12}C-Phe) dilution technique, which is described, to estimate the relative pool of phenylalanine (Phe) in the PMS of sham-irradiated and X-irradiated fetal rats. It demonstrates that the Phe pool in the fetal PMS is unaffected by irradiation 24 hours postexposure and indicates that the diminished incorporation of ^{14}C-labeled phenylalanine (^{14}C-Phe) into protein is the result of diminished protein synthesis in the PMS fetal cell-free system.

Materials and Methods

Pregnant Sprague-Dawley rats in their sixth day of gestation (day of conception = day zero) were obtained from a commercial source. Animals were either sham-irradiated or exposed to 250 kVcp X rays on day 13 of gestation. The sham- and X-irradiation was performed simultaneously on experimental animals and their controls. The X-irradiated animals received a total dose of 180 rads at a dose rate of 83 rads per minute. Animals were exposed in lucite containers placed 50 cm from the X-ray source which was filtered with 0.5 mm copper and 1.0 mm aluminum. The half value layer was 0.53 mm in copper. All animals were allowed food and water ad libitum up to the time of sacrifice.

Twenty-four hours after exposure to ionizing radiation pregnant animals were sacrificed, the uteri rapidly removed and placed in cold (4° C) tris-HCl buffer (100 mM tris-(hydroxymethyl)-aminomethane, 5 mM magnesium chloride, 150 mM sucrose, 2.5 mM potassium chloride, 5 mM 2-mercaptoethanol, adjusted to pH 7.3 with HCl). The fetuses were removed from the uteri, and mean fetal weight was determined for each litter.

PMS Cell-Free System

The various elements of the complete PMS cell-free system, in which the incorporation of a ^{14}C-labeled precursor amino acid into protein was measured, were prepared as follows (unless otherwise noted, all procedures were carried out at 4° C):

PMS - Fetuses obtained from pregnant mothers at the time of sacrifice were homogenized with ten strokes in a Dounce homogenizer using 1.7 volumes of the tris-HCl buffer. The homogenates were centrifuged at 15,000 G for 15 min. The lipid layer was removed and the clear post-mitochondrial supernatant (PMS) was obtained for use in the fetal PMS cell-free system. It should be noted that these studies employ a PMS based cell-free system.

Solution A_1 - An aqueous solution was prepared which was 0.2 mM with respect to each of the following L-amino acids: alanine, arginine (hydrochloride), aspartic acid, cysteine (sulfinic acid hydrate). glycine (ammonium free), glutamine (ammonium free), histidine (hydrochloride hydrate), hydroxy-proline, isoleucine (allo free), leucine, lysine (monohydrochloride), methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. In the preparation of this solution, the specific amino acid to be used as the labeled precursor in the PMS cell-free system was omitted.

Solution A_2 - An aqueous solution with the following composition: 400 mM tris-(hydroxymethyl)-aminomethane, 40 mM magnesium acetate, 200 mM potassium chloride, 24 mM 2-mercaptoethanol, 0.1 mM guanosine-5'-triphosphate (trisodium pentahydrate), 0.1 mM cytidine-5'-triphosphate (disodium hexahydrate), 0.1 mM uridine-5'-triphosphate (disodium hexahydrate), 0.4 mM glutathione (reduced). This solution was adjusted to pH 7.3 with HCl prior to dilution to final volume.

Solution A - This solution was prepared by combining equal volumes of Solutions A_1 and A_2.

Solution B - An aqueous solution containing the following in a final volume of 100 ml: 295 mg adenosine-5'-triphosphate (disodium trihydrate), 492 mg 2-phosphoenolpyruvic acid (tricyclohexylamonium salt), 492 enzyme units (E.U.) of pyruvate kinase (rabbit muscle, ammonium sulfate, specific activity 272 E.U. per mg), 24.6 ml of tris-HCl buffer (pH 7.3), and 49.2 ml of Solution A.

The uniformly labeled ^{14}C-amino acid to be used as a precursor of fetal protein was introduced into this solution prior to dilution to final volume. The quantities introduced for ^{14}C-phenylalanine were 133 μCi, specific activity 2.42 μCi per μg; for ^{14}C-lysine, 100 μCi, specific activity 0.62 μCi per μg.

Complete PMS Cell-Free System

This system was prepared by combining 0.75 ml of the PMS and 0.65 ml of Solution B. Three tenths ml of the complete PMS cell-free system was pipetted into each of three test tubes containing 0.2 ml of distilled water. The tubes were incubated with shaking in a 37° C water bath for 30 minutes. Incorporation of labeled amino acid into protein was terminated by the addition of 4 ml of 10 percent TCA to the incubation mixture. After 20 minutes at 4° C the precipitated incubation mixture was centrifuged and the pellets washed twice with 4 ml of 10 percent TCA. The second wash was heated in a 90° C water bath for 20 minutes and cooled to 4° C for 10 minutes prior to centrifugation. The pellets were then washed with alcohol:ether (3:1 by vol.), anhydrous ether, and air dried. The dried pellets were dissolved in 1 ml of concentrated formic acid and aliquots counted in Bray's Scintillant using a liquid scintillation spectrometer. Corrections for variable counting efficiency were made. Incorporation of ^{14}C-Phe into protein is expressed as dpm per g fetus. However, it should be noted that only the post-mitochondrial supernatant was used for the cell-free system. Thus, incorporation is measured in an amount of post-mitochondrial supernatant associated with the specified weight of fetal tissue.

Stable Isotope Dilution Technique for the Determination of Protein Synthesis and Fetal Phenylalanine in the PMS Cell-Free System

To obtain an insight into protein synthesis in control and X-irradiated fetuses from data on the cell-free incorporation of labeled phenylalanine into protein it was necessary to obtain estimates of the fetal precursor phenylalanine present in the PMS cell-free system. A technique was employed which measured ^{14}C-Phe incorporation in a given PMS cell-free system in which the initial amount of stable phenylalanine present in the cell-free system was artificially increased in a stepwise manner, all other constituents of the cell-free system being kept constant. The net effect of this increase in stable phenylalanine was to decrease the specific activity of the labeled precursor amino acid (AA) in the cell-free system at the start of the period of incubation ($t = 0$).

If we make the simplifying assumptions that:

1) the specific activity of precursor phenylalanine is constant during the time of incubation of the cell-free system when incorporation is measured, and

2) the amount of synthesis of protein during the period of incubation is unaffected by the deliberate increases in the amount of stable phenylalanine present in the cell-free system at $t = 0$, we can write:

$$\text{Incorporation} = \frac{{}^{14}\text{C-AA}}{AA_1 + AA_2 + AA_3} \cdot K\ , \tag{1}$$

in which

$\dfrac{{}^{14}\text{C-AA}}{AA_1 + AA_2 + AA_3}$ is the specific activity of the labeled precursor AA in the cell-free system during the period of incubation, and

K is the amount of precursor AA incorporated into protein during the period of incubation.

At $t = 0$:

^{14}C-AA is equal to the amount of labeled precursor AA introduced into the cell-free system, expressed in dpm.

AA_1 is equal to the precursor AA associated with the labeled AA introduced into the cell-free system, expressed in µg.

AA_2 is equal to the amount of stable precursor AA from the fetal post-mitochondrial supernatant present in the cell-free system, expressed in µg.

AA_3 is equal to the amount of stable precursor AA added in order to deliberately depress the specific activity of the labeled AA precursor of protein in the cell-free system prior to incubation.

All of the terms in equation 1 are either known or measured with the exception of AA_2 and K. By using two or more values of AA_3 to measure incorporation in otherwise identical cell-free systems, it is possible to solve for both AA_2 and K.

Given that incorporation (Inc) is measured with two $t = 0$ values of AA_3, AA_{3i} and AA_{3f}, in which $AA_{3f} > AA_{3i}$, we can write:

$$AA_2 = \frac{\text{Inc}_f\, AA_{3f} - \text{Inc}_i\, AA_{3i} - AA_1\ (\Delta\ \text{Inc})}{\Delta\ \text{Inc}} \tag{2}$$

Inc_i and Inc_f are measured when $AA_3 = AA_{3i}$ and AA_{3f}, respectively, thus $\text{Inc}_i > \text{Inc}_f$. $\Delta\ \text{Inc} = \text{Inc}_i - \text{Inc}_f$.

When $AA_{3i} = 0$

$$AA_2 = \frac{Inc_f \; AA_{3f} - AA_1 \; (\Delta \; Inc)}{\Delta \; Inc} \tag{3}$$

Once AA_2 is known, K can be computed using equation 1.

If we take the reciprocal of equation 1 and rearrange terms we obtain:

$$\frac{1}{Inc} = \frac{AA_2}{(^{14}C\text{-}AA)} + \frac{1}{(^{14}C\text{-}AA)} (AA_1 + AA_3) \tag{4}$$

This is an equation for a straight line (Figure 63) with the y intercept $\frac{AA_2}{(^{14}C\text{-}AA)K}$, the x intercept AA_2 and the slope $\frac{1}{(^{14}C\text{-}AA)K}$. The slope of $\frac{1}{Inc}$ as a function of $AA_1 + AA_3$ can thus effectively be $\frac{1}{K}$ · constant if experimental techniques are used that control variation of $\frac{1}{^{14}C\text{-}AA}$. The slope of $\frac{1}{Inc}$, under such conditions, can be used to assess possible changes in the amount of synthesis observed in cell-free systems in which two experimental treatments have been employed. Further, the linearity of $\frac{1}{Inc}$ vs. $(AA_1 + AA_3)$ can be used as a test of the effect or absence of an effect of increased AA_3 on the amount of protein synthesis observed in the cell-free system during the period of incubation.

When $AA_1 + AA_3 = 0$, then:

$$\frac{1}{Inc} = \frac{AA_2}{(^{14}C\text{-}AA)K} . \tag{5}$$

This is the y intercept of the line described by equation 4.

When $AA_1 + AA_3 = AA_2$, then:

$$\frac{1}{Inc} = 2 \; \frac{AA_2}{(^{14}C\text{-}AA)K} . \tag{6}$$

Thus when the amount of $AA_1 + AA_3$ is equal to the amount of AA_2 in the PMS cell-free system, $\frac{1}{Inc}$ will attain a value which is twice that of the y intercept, i.e., incorporation measured in the cell-free system will be reduced to one-half that observed when $(AA_1 + AA_3) = 0$.

While the techniques described in equations 1-4 have been used for the amino acid phenylalanine in this cell-free system it should be apparent that this approach may be applied in any similar precursor-compound situation where it may be either convenient or necessary.

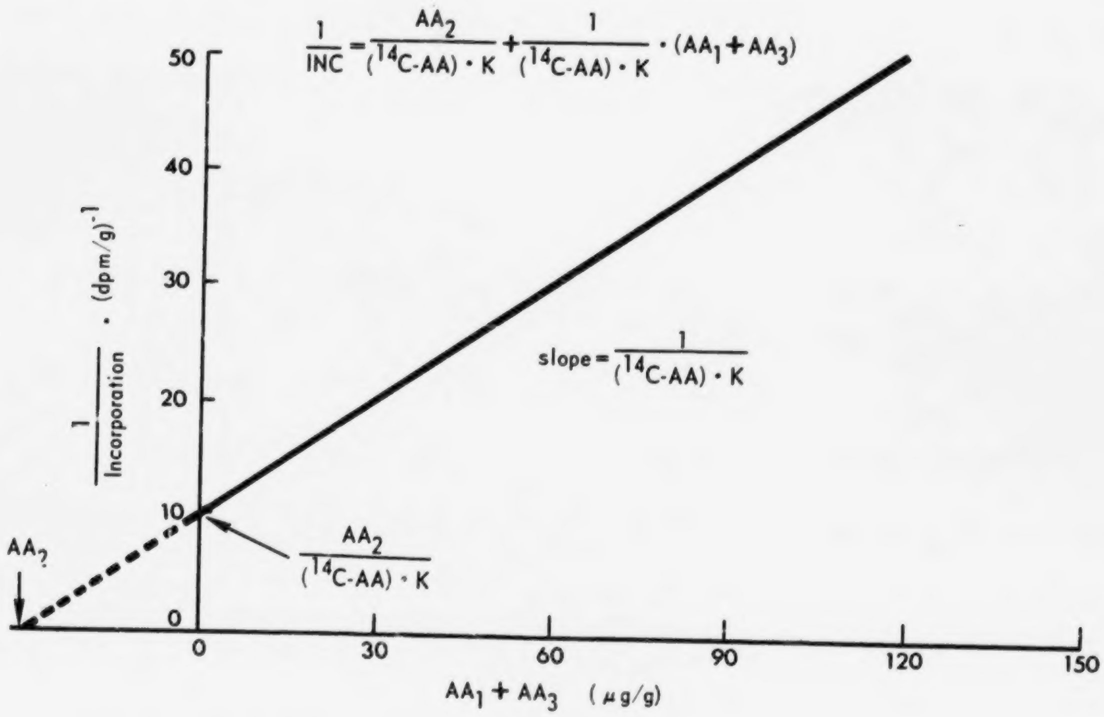

Figure 63. Theoretical Plot of $\frac{1}{Inc}$ Vs. $AA_1 + AA_3$ in the Described Model of Cell-Free Protein Synthesis.

Results and Discussion

In the initial series of experiments, an effort was made to determine whether the incorporation of ^{14}C-AA into protein would be affected by increased concentrations of AA within the range of interest, in the cell-free system. Incorporation of ^{14}C-lysine was measured in the presence of increased concentrations of Phe. Additionally, incorporation of ^{14}C-Phe was measured in the presence of increased tryptophan and tyrosine. Since it was also of primary interest to obtain information on the effect of fetal X-irradiation on incorporation of ^{14}C-AA into protein, these studies employed cell-free systems from fetuses X-irradiated in utero 24 hours prior to sacrifice. The dose of radiation employed was 180 rads delivered to pregnant rats on day 14 of gestation.

Table 23-A presents data in which ^{14}C-lysine incorporation was measured in the presence of t = 0 levels of Phe, expressed as µg/g fetus, ranging from AA_3 = 0 to AA_3 = 340 µg/g. It can be seen that increased levels of Phe within the range tested appeared to be without effect on the incorporation of ^{14}C-lysine into the acid insoluble fraction.

Tables 23-B and 23-C present data in which the incorporation of ^{14}C-Phe into protein was measured in the presence of increased tryptophan and tyrosine. Increased amounts of these amino acids in the cell-free system at t = 0, within the limits tested, appeared to be without effect on the incorporation of ^{14}C-Phe into protein.

TABLE 23. EFFECT OF INCREASED AA_3 ON THE INCORPORATION OF ^{14}C-AA INTO PROTEIN IN CELL-FREE SYSTEMS DERIVED FROM SHAM- AND X-IRRADIATED FETUSES 24 HOURS AFTER IN UTERO IRRADIATION

Treatment	AA_3 = 0	AA_3 = 170 µg/g	AA_3 = 340 µg/g
Incorporation x 10^{-3} (dpm/g) of ^{14}C-lysine with Increased Phe (AA_3)			
Sham-Irradiated	19.5 22.6	24.7 25.7	20.5 25.5
X-Irradiated	15.8 15.4	17.1 12.9	16.3 15.8
Incorporation x 10^{-3} (dpm/g) of ^{14}C-Phe with Increased Tryptophan (AA_3)			
Sham-Irradiated	59.2 58.4	63.6 61.9	
X-Irradiated	38.2 39.2	39.9 35.4	
Incorporation x 10^{-3} (dpm/g) of ^{14}C-Phe with Increased Tyrosine (AA_3)			
Sham-Irradiated	65.4 61.5 64.7 65.7	64.3 63.5 -- --	61.8 61.1 -- --
X-Irradiated	46.2 43.9 50.1 46.5	42.8 43.7 -- --	50.3 47.4 -- --

Examination of the data in Table 23 for the effect of X-irradiation on ^{14}C-AA incorporation into protein in the PMS cell-free system shows, in every case, that exposure to radiation was associated with reduced incorporation of ^{14}C-AA into protein, regardless of $t = 0$ levels of AA_3. This finding agrees with our previously reported observations.

TABLE 24. WEIGHT OF SHAM- AND X-IRRADIATED FETUSES 24 HOURS AFTER EXPOSURE.

Sham-Irradiated		X-Irradiated	
No. Fetuses Litter	Fetal Wet Weight (g)	No. Fetuses Litter	Fetal Wet Weight (g)
6	0.134	12	0.118
13	0.148	13	0.123
12	0.149	13	0.127
11	0.128	11	0.135
10	0.140	11	0.115
11	0.141	13	0.094
10	0.151	10	0.123
		10	0.123
$\bar{X}$	0.142		0.120
S.E.	0.003		0.004

Table 24 presents data on the weight of the sham- and X-irradiated fetal rats employed in this study. The weight of X-irradiated fetuses was significantly less than that of sham-irradiated controls. This is consistent with our previous observation of the stunting effect of X irradiation on the fetal rat 24 hours after exposure to ionizing radiation. The decrease in incorporation of labeled amino acids in irradiated fetal rats (Table 23) was measured in a volume of post-mitochondrial supernatant associated with a unit weight of fetal tissue. Thus, the impact of radiation on incorporation of labeled amino acid into protein per total fetus is greater than that shown in

Table 23 by a factor quantitatively related to the stunting effect on irradiated fetuses.

In the following series of experiments, pooled fetal PMS was used to prepare PMS cell-free systems in which ^{14}C-Phe incorporation was measured as a function of decreased specific activity of precursor Phe (i.e., increasing $AA_1 + AA_3$ with ^{14}C-AA relatively constant). Four litters of fetuses were homogenized for the preparation of each lot of pooled PMS. Four lots of PMS were prepared from sham-irradiated fetuses, and four lots from X-irradiated fetuses.

PMS cell-free systems were prepared from each lot of PMS with six values of $AA_1 + AA_3$ ranging from 1.16 to 103 μg/g (expressed as μg Phe per g of fetus). At each value of $AA_1 + AA_3$ duplicate PMS cell-free systems were prepared in which ^{14}C-Phe incorporation into protein was separately determined for each system.

Table 25 presents the data that were obtained for incorporation of ^{14}C-Phe in cell-free systems from both sham-irradiated and X-irradiated fetuses.

A test was performed to determine whether the data obtained conformed to the linear model described by equations 1 and 4 (Materials and Methods).

The fit to the data from the four sham-irradiated cell-free systems is presented in Figure 64 and is described, in the form of equation 4, by the following:

$$\frac{1}{\text{Inc}} = [16.0 \times 10^{-6} + 0.403 \times 10^{-6} \, (AA_1 + AA_3)] \, (\tfrac{\text{dpm}}{\text{g}})^{-1} \qquad (7)$$

The data obtained did not deviate significantly the linearity defined by equation 7. In the form of equation 1 the data obtained fits the following equation:

$$\text{Inc} = \frac{6.48 \times 10^{6} \text{ dpm/g}}{39.7 \frac{\mu g}{g} + (AA_1 + AA_3)} \qquad 0.383 \ \mu g/g \qquad (8)$$

The fit to the data for X-irradiated cell-free systems is presented in Figure 64 and is described by the following equation:

$$\frac{1}{\text{Inc}} = [20.9 \times 10^{-6} + 0.607 \times 10^{-6} \, (AA_1 + AA_3)] \, (\tfrac{\text{dpm}}{\text{g}})^{-1} \qquad (9)$$

The data obtained did not deviate significantly from the linearity defined by equation 9.

TABLE 25. DECREASING INCORPORATION (INC) OF LABELED PHE (^{14}C-AA) INTO PROTEIN AS A FUNCTION OF INCREASED STABLE PHE (AA_3) IN THE PMS CELL-FREE SYSTEM FROM SHAM- AND X-IRRADIATED FETAL RATS.

1		2		3		4	
Sham-Irradiated							
^{14}C-AA = 6.14 x 10^6 dpm AA_1 = 1.16 μg/g		^{14}C-AA = 6.60 x 10^6 dpm AA_1 = 1.17 μg/g		^{14}C-AA = 6.69 x 10^6 dpm AA_1 = 1.16 μg/g		^{14}C-AA = 6.48 x 10^6 dpm AA_1 = 1.16 μg/g	
Inc x 10^{-3} dpm/g	AA_3 μg/g	Inc x 10^{-3} dpm/g	AA_3 μg/g	Inc x 10^{-3} dpm/g	AA_3 μg/g	Inc x 10^{-3} dpm/g	AA_3 μg/g
58.9	0	63.4	0	65.2	0	69.5	0
33.2	33.5	29.9	33.8	43.1	16.8	49.9	16.8
27.5	50.3	23.9	50.7	30.1	33.6	35.4	33.5
24.6	67.1	22.0	67.6	24.5	50.5	28.2	50.3
21.1	83.9	18.0	84.5	22.4	67.3	24.0	67.1
19.5	101.	17.2	101.	16.6	101.	21.8	83.9
X-Irradiated							
^{14}C-AA = 6.14 x 10^6 dpm AA_1 = 1.16 μg		^{14}C-AA = 6.60 x 10^6 dpm AA_1 = 1.17 μg		^{14}C-AA = 6.74 x 10^6 dpm AA_1 = 1.17 μg/g		^{14}C-AA = 6.48 x 10^6 dpm AA_1 = 1.16 μg/g	
Inc x 10^{-3} dpm/g	AA_3 μg/g	Inc x 10^{-3} dpm/g	AA_3 μg/g	Inc x 10^{-3} dpm/g	AA_3 μg/g	Inc x 10^{-3} dpm/g	AA_3 μg/g
38.7	0	45.1	0	48.3	0	44.9	0
21.7	33.5	19.9	33.8	34.8	17.0	34.4	16.8
18.5	50.3	18.5	50.7	25.7	33.9	26.1	33.5
15.0	67.1	15.4	67.6	20.6	50.9	21.6	50.3
12.4	83.9	13.7	84.5	17.7	67.8	17.1	67.1
11.0	101.	11.9	101.	12.6	102.	15.6	83.9

In the form of equation 1 the data fit the following equation:

$$\mathrm{Inc} = \frac{6.49 \times 10^{6}\ \mathrm{dpm/g}}{34.4\ \frac{\mu g}{g} + (AA_1 + AA_3)} \cdot 0.254\ \mu g/g \qquad (10)$$

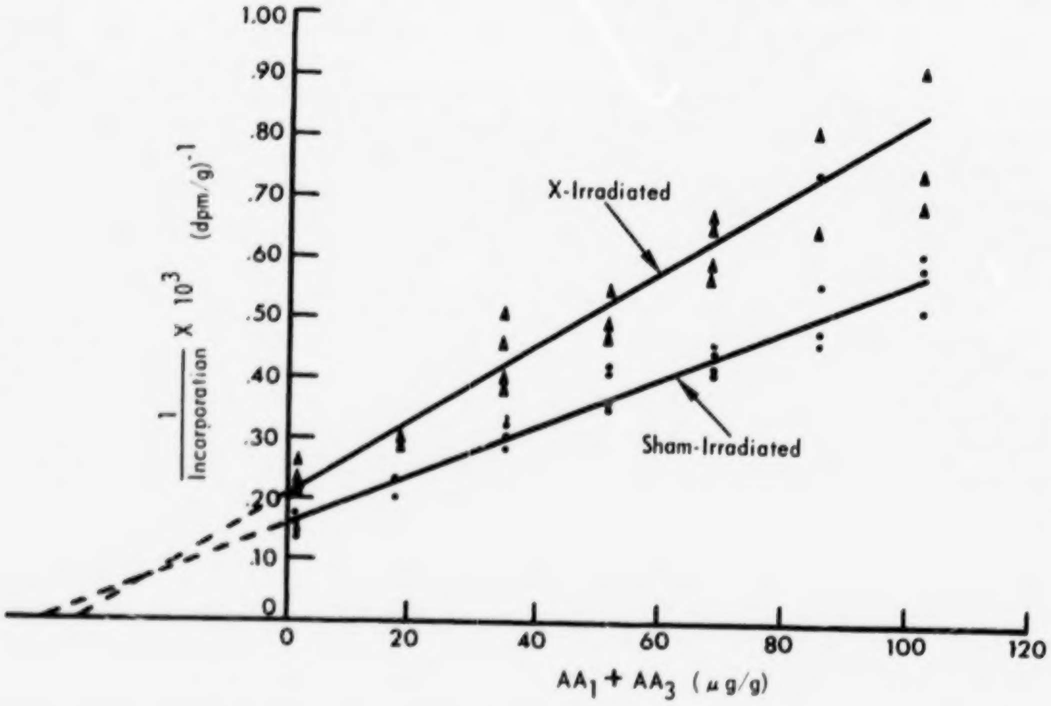

Figure 64. Least Squares Fit of Data on $\frac{1}{\mathrm{Inc}}$ Vs. $AA_1 + AA_3$ Obtained from Cell-Free Systems of Sham- and X-Irradiated Fetuses.

The lack of significant deviation of the data from linearity described by equations 7 and 9 suggests that the simplifying assumptions that underlie the model of cell-free protein synthesis used in this study are tenable. Thus, it appears that (1) the specific activity of precursor phenylalanine can be considered to be constant during the period of incubation when incorporation is measured, i.e., protein degradation does not add significant amounts of stable Phe to the precursor AA pool during the period of incubation, and (2) the amount of synthesis of protein during the period of incubation is unaffected by the increases in the amount of ^{12}C-Phe present in the cell-free system at t = 0 (for the range of $AA_1 + AA_3$ used in this study). The latter conclusion is in agreement with the data presented earlier showing that increased amounts of specific stable AA appear to be without effect on the incorporation of other labeled precursor AA into protein in the PMS cell-free system, within the range of values employed in this study.

The test of the slopes $1/(^{14}C\text{-Phe})K$ (Figure 64) for sham-irradiated and X-irradiated cell-free systems indicated that these were significantly different ($P < .05$), with the slope for the X-irradiated animals > slope for the sham-irradiated animals. Since the slope of 1/Inc approximates $\frac{1}{K} \cdot$ constant in these experiments, finding this suggests that the amount of protein synthesized during the period of incubation in the X-irradiated cell-free systems is significantly less than that synthesized in the sham-irradiated control systems. The estimated value of K (equations 8 and 10) for the irradiated animals was 0.254 μg/g, and for the sham-irradiated controls it was 0.383 μg/g (expressed in μg/g fetus).[1]

Estimates of the fetal pool of phenylalanine in the cell-free system (AA_2) were made. AA_2, the (AA_1+AA_3) intercept of 1/Inc, was computed from the least squares fits of the data obtained from sham-irradiated and X-irradiated cell-free systems and is presented in Table 26 [$(AA_2)_1$]. The estimate $(AA_2)_1$ is a least squares estimate and weights all of the data on which it is based equally. No significant difference in the pool of fetal phenylalanine in the cell-free system was observed between sham-irradiated and X-irradiated animals.

Alternate estimates of the fetal pool of Phe in the PMS cell-free system were computed [$(AA_2)_2$] and these are presented in Table 26. These estimates were obtained using equation 3. The incorporation of ^{14}C-Phe into protein without any addition of stable phenylalanine, i.e., $AA_3 = 0$, was paired with incorporation at each of the 5 additional levels of AA_1+AA_3 at which incorporation was measured. Each pair of values was used to compute 5 estimates of AA_2 and these were averaged to obtain each of the tabulated estimates of fetal Phe in the cell-free system, $(AA_2)_2$. The mean pool of fetal Phe in the cell-free system for sham-irradiated fetuses was 36.2 ± 3.2 μg/g fetus. For X-irradiated fetuses the value was 39.5 ± 2.6 μg/g fetus. These means are not significantly different ($P > .05$). It should be noted that this method for calculating $(AA_2)_2$ assigns a weight of 50 percent to the value for incorporation without added Phe (i.e., $AA_3 = 0$). The values of the means for the estimates of AA_2 obtained by both of these methods, $(AA_2)_1$ and $(AA_2)_2$, are not significantly different. It is apparent from equations 2 and 3 that two points, accurately determined, would permit calculation of the value of fetal precursor AA in the cell-free system (AA_2).

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[1] These are approximations since $1/^{14}C$-Phe was relatively but not absolutely constant in these experiments.

TABLE 26. ESTIMATES OF THE FETAL PHENYLALANINE POOL (AA_2) AND THE AMOUNT OF PROTEIN SYNTHESIS (K) IN THE CELL-FREE SYSTEM FROM SHAM- AND X-IRRADIATED FETAL RATS.

Treatment	Least Squares Estimate[a] (AA_2)μg	Weighted Estimate[b] (AA_2)μg	$K^c_{\mu g}$
	Sham-Irradiated		
1	51.4	45.3	0.445
2	41.4	32.5	0.324
3	36.7	31.1	0.315
4	36.2	36.0	0.399
$\bar{X}$	39.7	36.2	0.371
S.E.	4.7	3.2	0.013
	X-Irradiated		
1	36.2	41.6	0.270
2	43.2	33.0	0.234
3	33.1	37.9	0.280
4	39.8	45.4	0.324
$\bar{X}$	34.4	39.5	0.277
S.E.	4.9	2.6	0.019

a. Least Squares Estimate - These values are unbiased estimates of AA_2 computed from the least squares fits of the data presented in Table 26. They have the same absolute value as ($AA_1 + AA_3$) when 1/Inc = 0 and the value of ($AA_1 + AA_3$) when 1/Inc = $\frac{2\ AA_2}{(^{14}\text{C-Phe})K}$ (Fig.63)

b. Weighted Estimate - These values are calculated in the following manner: AA_2 is computed using equation 3 and 5 pairs of values for 1/Inc and AA_3 one of which is always the value for 1/Inc when $AA_3 = 0$. The five values for AA_2 which are calculated are averaged to obtain each value of (AA_2).

c. K - These values are computed from equation 1 using the weighted estimate of (AA_2) and the value of incorporation observed without addition of stable phenylalanine (i.e., $AA_3 = 0$).

Using the values of $(AA_2)_2$, and equation 1 the values of K were computed and are presented in Table 26. K represents the amount of phenylalanine, expressed in μg/g fetus, incorporated into protein during the period of incubation of the fetal PMS cell-free system. The value of K for sham-irradiated fetuses was 0.371 ± 0.031 μg/g. This compares with the previously estimated value of 0.383 μg/g (equation 8). The value for X-irradiated fetuses was 0.277 ± 0.019 μg/g. This compares with the previously estimated value of 0.254 μg/g (equation 10). The values of K for X-irradiated fetuses are significantly lower than those for sham-irradiated fetuses. Thus X-irradiation of fetuses was associated with a significant decrease in the incorporation of phenylalanine into fetal protein.

The present work indicates that fetal X-irradiation on day 13 of gestation is associated with a decrease in the body weight of irradiated fetuses 24 hours after exposure. Fetal incorporation of labeled amino acid into protein, based on observations using the PMS cell-free system, is reduced by irradiation. X-irradiation on day 13 of gestation does not appear to alter the amount of fetal Phe in the PMS of irradiated fetuses 24 hours after exposure. The decrease in the incorporation of ^{14}C-Phe observed in irradiated fetuses 24 hours after exposure results from a decrease in the amount of PMS cell-free protein synthesis.

The results of this study are consistent with our previous observation that fetal irradiation is associated with decreased synthesis of liver enzymes in newborn rats (2); our observation of impaired incorporation of ^{14}C-Phe into protein in vivo and in vitro in fetally irradiated rats 24-72 hours after exposure (3, 4); and our observation that irradiation is associated with decreased induction of tryptophan pyrrolase in adult rats when the induction of this enzyme is dependent on the synthesis, after irradiation, of messenger RNA (5). The mechanisms underlying decreased protein synthesis in fetuses following X-irradiation require further investigation.

---

[2] Smith, C. H. and M. L. Shore. 1966. Radiation Res. 29:499.

[3] Shore, M. L., J. W. Laskey, L. R. Simmons, G. L. Jessup, and K. M. Selby. p.

[4] Laskey, J., L. Simmons, K. Selby, and M. L. Shore. p.

[5] Mishkin, E. P. and M. L. Shore. 1968. Radiation Res. 33:437.

Summary

Irradiation on day 13 of gestation is associated with a measurable stunting effect in fetal rats 24 hours after exposure.

Using a PMS cell-free system, which is described, it was found that incorporation of ^{14}C-labeled amino acids into fetal protein was depressed in fetal rats 24 hours after exposure to X rays.

A stable isotope dilution technique was employed to obtain estimates of the amount of precursor fetal phenylalanine present in the fetal cell-free system. No differences were observed in fetal PMS phenylalanine pool between sham- and X-irradiated fetuses 24 hours after exposure.

The data obtained using the PMS cell-free system indicate that protein synthesis is depressed in irradiated fetuses.

DOSIMETRIC STUDIES SECTION

Mr. George E. Anderson, Chief

The Dosimetric Studies Section provides an irradiation facility and the supportive dosimetry for the Division of Biological Effects. Associated research studies are conducted in the related areas of dosimetry. In addition, the Section provides services in the fields of electronics, bioengineering, and physics.

Low Energy X Ray

A low-energy X-ray unit was completed and calibrated during 1969. This specially constructed X-ray unit, which can emit X rays similar to those emitted by color television receivers, permits the Bureau to study biological effects of low energy photons. The addition of this machine allows the Section to provide peak kilovoltage settings from 10 to 120, at up to 100 millamperes, with a wide range of filtration.

The problem of simulating the color television X-ray spectra was solved, enabling 260 biologically related exposure periods. The associated dosimetry for these projects has been nearly completed.

Microwaves

A small, temporary microwave anechoic chamber was constructed to provide a microwave exposure laboratory for biological specimens. The initial exposure source was a microwave oven which had been modified to operate with the door removed. Subsequently, much more rigid specifications were developed, and an industrial microwave generator was purchased. This unit was used with a standard gain horn to provide the exposure field. The source output was 2.5 kilowatts of CW transmitted power at 2450 megahertz. Both sources were used for biological exposure; operation of the oven was discontinued since it proved to be unreliable.

The original anechoic chamber failed to meet the expanding needs of the Division because of its inadequate size. A new, much larger chamber and exposure area was fabricated with material (Emerson & Cummings CVB-24 and CVB-24F) acquired from the Goddard Space Flight Center. The new design will permit far field measurements and exposures at up to 30 feet source-to-sample distance. An environmental control and data acquisition system is expected to be incorporated in the microwave exposure facility during 1970. Preliminary work was done on system requirements and performance specifications. It will provide a means of controlling the animal's environment as it is exposed to microwave radiation and allow monitoring of the various physiological parameters.

The further development of exposure field mapping techniques is projected for 1970. Spatial and temporal profiles of the macroscopic temperature of biological equivalent phantoms will be studied. This will provide insight into the problem of determining energy density distributions.

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A. ENERGY ABSORPTION BY DIELECTRICS IN A CAPACITOR

Dan Dawes

The volume occupied by the dielectric between the plates of a capacitor offers a means of experimentally measuring microwave power absorption. To evaluate the power density, the field between the plates of a capacitor was computed and compared to a plane wave, free-space exposure.

A circular capacitor having radius $b \ll \lambda$, a good but imperfect dielectric completely filling the volume between the plates, a plate separation, $w \ll \lambda$, and plates of infinite conductivity was analyzed. The capacitor was assumed to have negligible fringing. The fields were found to be

$$E_z(r) = \frac{1}{\sigma + i\omega\varepsilon}\left(\frac{I_z}{\pi b^2}\right)\left(\frac{xb}{2}\right)\frac{J_0(xr)}{J_1(xb)}$$

$$B_\theta(r) = \frac{\mu b}{2}\left(\frac{I_z}{\pi b^2}\right)\frac{J_1(xr)}{J_1(xb)}$$

where:

$x^2 = \omega^2\mu\,(\varepsilon - i\sigma/\omega)$
B_θ = Flux density in azimuthal direction in weber/meter^2
σ = Conductivity in mho/meter
ε = Dielectric constant in farads/meter
E_z = Electric field in the z direction in volts/meter
μ = Permeability in henrys/meter
ω = Angular frequency in radians/second
J_1 and J_0 = First and zeroth order Bessel functions
I_z = Current flowing through the capacitor in amperes
r = Radius of capacitor in meters in cylindrical coordinates

The time averaged power absorbed in the entire volume of dielectric was found to be

$$P \approx \frac{\tan\delta}{\omega\varepsilon(1 + \tan^2\delta)}\;\frac{\langle I_z^2\rangle}{2\pi b^2}\,w$$

where $\langle I_z^2\rangle$ is the mean square of the current, and $\tan\delta$, the loss tangent. This can be compared to the power absorbed by the same dielectric in a plane wave exposure field (see p.

Figure 65 is a graph of the relative power absorption with distilled water as the dielectric at 1 MHz.

A capacitor's field could possibly be profitably used for frequencies lower than 1 MHz for small samples in carefully designed apparatus. It affords the advantages of economy and a means of experimentally measuring whole-body power absorption. However, far-field free space exposure is seen as the best present mode of irradiation for high frequencies and large targets. Figure 66 pictures a model of the time averaged power density at 2450 MH_z for a free space exposure.

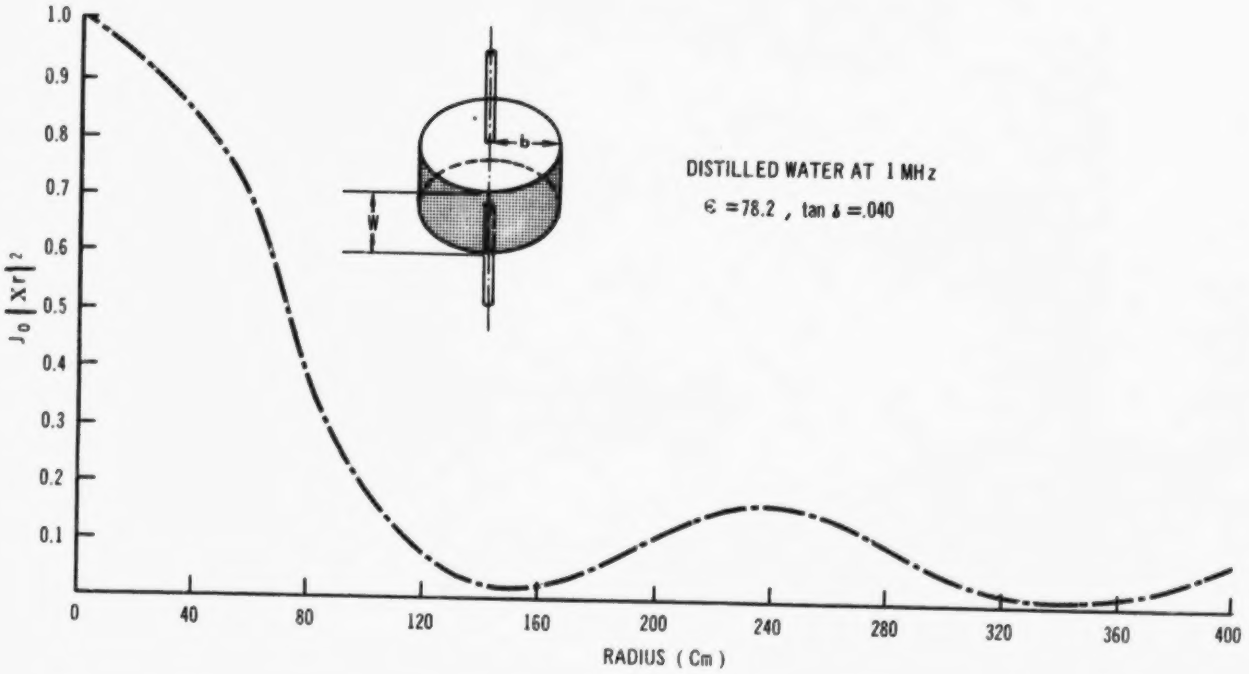

Figure 65. Average Power Density Vs. Radial Distance in a Capacitor Having Radius b and a Dielectric of Thickness W.

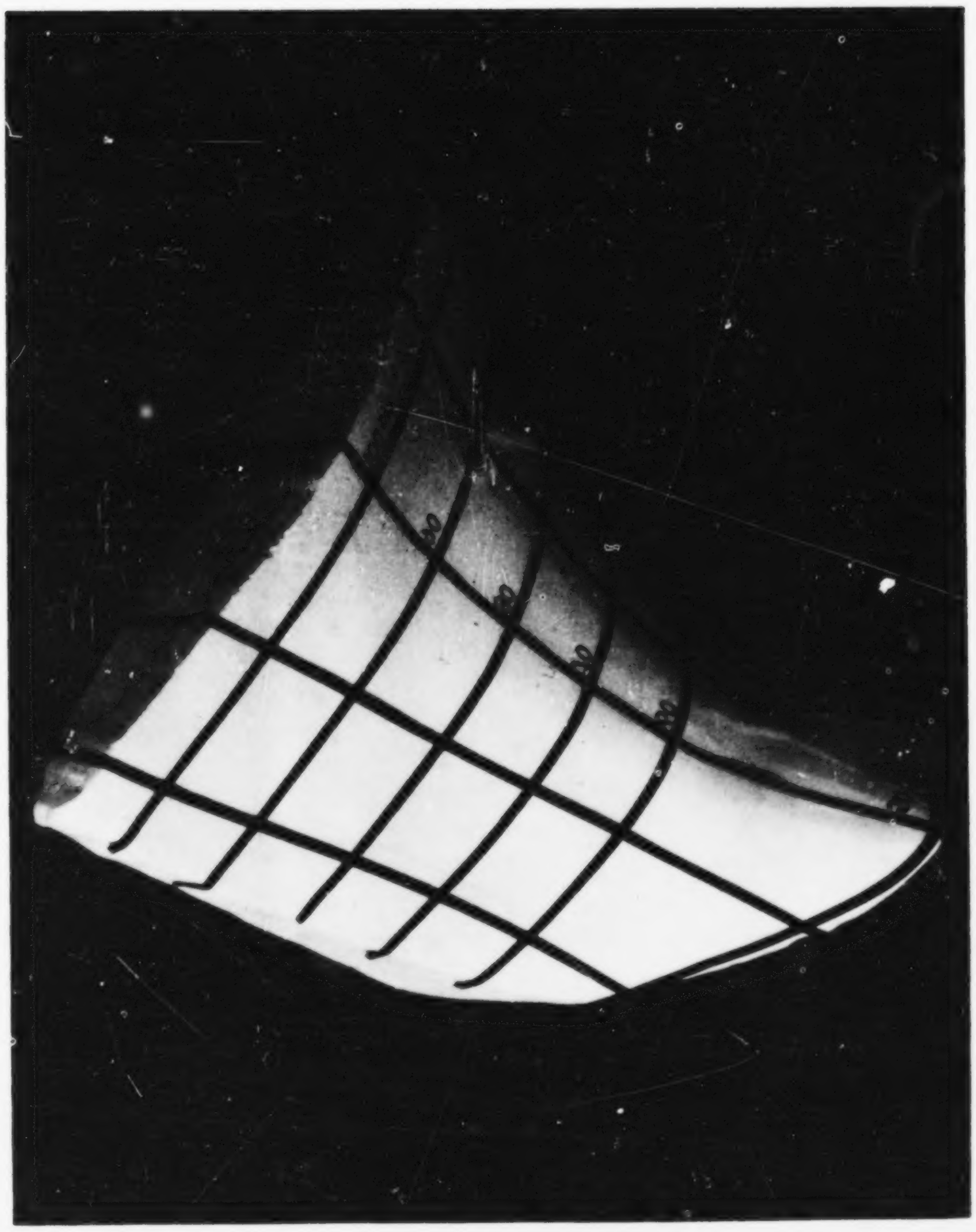

Figure 66. Plaster Model Showing Average Power Density in the H Plane from a 10.25 dB Standard Gain Pyramidal Horn at a Distance of 50 to 100 cm. The X-Y scale is 1 inch to 10 cm, and the lateral edges are half-power points. Measurements were performed in an anechoic chamber using a tuned dipole and a PRD bolometer.

B. ELECTRICAL PROPERTIES OF COMMERCIAL INSULATORS

Dan Dawes, Lynda Kramer

In biological studies employing microwaves, it is imperative to know as closely as possible the energy absorbed by the specimen. Many specimens during irradiation are enclosed in containers that reflect, absorb, and transmit microwaves. To approximate these characteristics, the Section compiled extensive tables. The tables list the dielectric constant and loss tangent for common commercial insulators taken from existing tables.[1,2] A small portion of the tables is reproduced here as Table 27.

The attenuation in decibels per meter and the percentage of power reflected for transverse electromagnetic (TEM) waves normally incident on non-magnetic material has been calculated and listed, using the following relations (p. 28 and 53, A.R. von Hippel, Dielectrics & Waves).

$$\mathrm{dB/m} = \frac{8.6862\pi}{\lambda}\left[\tfrac{1}{2}\epsilon\left(\sqrt{1+\tan^2\delta}-1\right)\right]^{\frac{1}{2}}$$

$$R = |r_e|^2 = \left|\frac{1-\sqrt{\epsilon^*}}{1+\sqrt{\epsilon^*}}\right|^2$$

where:

λ = Free space wavelength
ϵ = Relative dielectric constant
δ = Loss angle
ϵ^* = Relative complex dielectric constant

Example of use:

> A flat sheet of plexiglass is irradiated by normally incident TEM waves at the power density of 100 mW/cm^2. The sheet is 1 cm thick. From the table, 5.5 percent of the energy is reflected. Of the 94.5 mW/cm^2 penetrating the first interface, 2.51 db/m or only 0.58 percent is dissipated in one pass through 1 cm of plexiglass, i.e., 0.548 mW/cm^2. Of the 94 mW/cm^2 incident on the rear face, about 5.5 percent is reflected or 5.16 mW/cm^2; the remaining 89 mW/cm^2 is transmitted entirely through. Of the 5.16 mW/cm^2 reflected off the back face, 0.03 mW/cm^2 is absorbed by the time the energy is partially re-reflected from the front interface. The process can be carried on indefinitely. Approximately then, of 100 mW/cm^2 incident, 89 mW/cm^2 is transmitted thru 1 cm, 0.6 mW/cm^2 is absorbed, 10.4 mW/cm^2 is reflected.

---

[1] Tables of Dielectric Materials, Vol. I-IV, Insulation Research of the M.I.T., Jan., 1953.

[2] Dielectric Materials and Applications by A.R. von Hipple, (ed.), John Wiley and Sons, N.Y.,1954.

The power absorbed in a semiinifinite sheet of thickness, w, due to exposure to a power flux[1] of P_o is

$$P = P_o \frac{(1 - R)(1 - 10^{(wa/10)})}{1 - R^{(wa/10)}}$$

where:

$$R = \left| \frac{1 - \sqrt{\varepsilon^*}}{1 + \sqrt{\varepsilon^*}} \right|^2$$

$$a = \frac{8.686\ 2\pi}{\lambda} \left[\tfrac{1}{2}\varepsilon \left(\sqrt{1 + \tan^2\delta} - 1\right) \right]^{\frac{1}{2}}$$

This expression takes an infinite number of reflections into account.

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[1] Only a normally incident TEM plane wave is considered.

TABLE 27. ELECTRICAL PROPERTIES OF COMMON INSULATORS

Trade Name	Material	T, $^{\circ}$C	3 GHz ε	 Tan δ	Loss, dB/meter	Reflection, percent
Corning 7060 (Pyrex)	Soda-potash borosilicate	25	4.82	0.0054	3.23	14.0
Quartz (fuzed)	Silicon dioxide	25	3.78	0.00006	0.031	10.3
Hydrogenated polystyrene	Polyvinyl-cyclohexane	24	2.25	0.00018	0.073	4.0
Nylon 610	Polyhexamethy-leneadipamide	25	2.84	0.0117	5.83	6.5
Plexiglass	Polymethyl-methacrylate	27	2.60	0.0057	2.51	5.5
Polystyrene	-	25	2.55	0.0033	0.143	5.3
Teflon	Polytetrafluoro-ethylene	22	2.1	0.00015	0.059	3.4
Ethyl Alcohol	Absolute	25	6.5	0.250	172.59	21.0
Vaseline	-	25	2.16	0.00066	0.264	3.6
Paraffin Wax 132° ASTM	C_{22} - C_{29} aliphatic, saturated hydrocarbons	25	2.25	0.0002	0.082	4.0
Ruby Mica	Muscovite	26	5.4	0.0003	0.190	15.9
Water	Distilled	25	76.7	0.157	374.02	63.4

C. TEMPERATURE CONTROLLER DESIGN FOR A DIGITAL GLOW-CURVE ANALYZER

Joseph S. Ali

Goals, Previous Work

In October, 1969, design of a temperature controller for a thermoluminescent dosimeter (TLD) reader was initiated. This temperature controller will be part of a digital glow curve analyzer, a special type of TLD reader (Figure 67). Other parts of this system have already been designed and built (specifically, the Current to Pulse Rate Converter)[1] and modifications to the Victoreen Skipp Multichannel Analyzer have been completed to accept the analog temperature sweep shown in Figure 67. This reader will be used to study the feasability of using calibrated TLD crystals to measure the microwave power level in free space and in biologically equivalent phantoms when irradiated in an exposure chamber. The TLD crystals will first be exposed to a known X-ray dose to calibrate the crystals. Then they will be encapsulated in a "lossy" dielectric material. These small spherical dosimeters will be exposed in free space or imbedded in phantoms and then exposed.

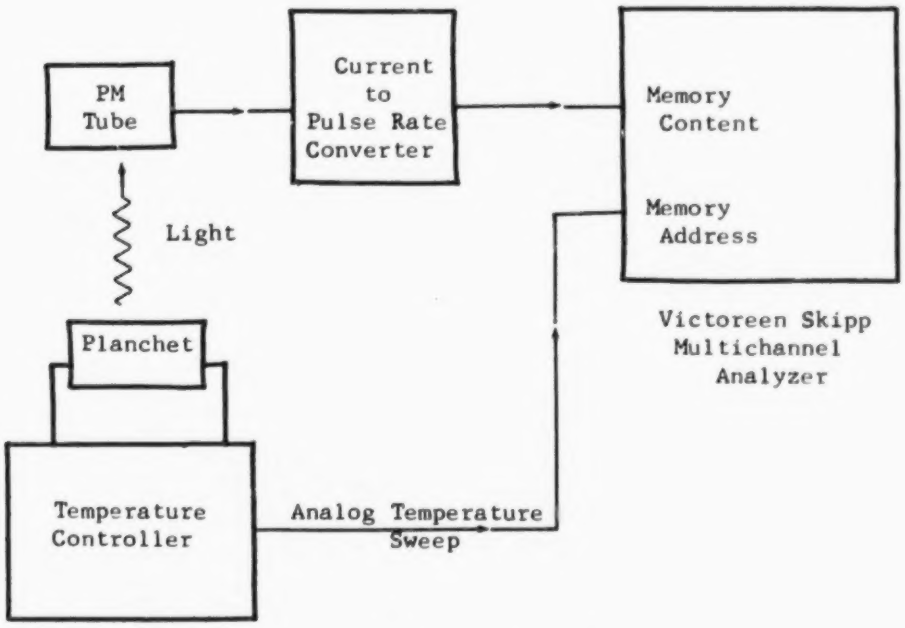
PM
Tube
Current
to
Pulse Rate
Converter
Memory
Content
Memory
Address
Light
Planchet
Victoreen Skipp
Multichannel
Analyzer
Temperature
Controller
Analog Temperature
Sweep

Figure 67. Digital Glow Curve Analyzer

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[1] Anderson, G.E., Analog-To-Digital Converter, p. 67-68, In Radiation Bio-Effects Summary Report, 1967, Bureau of Radiological Health.

When exposed to a microwave field, the temperature of the lossy dielectric will rise causing some of the energy stored in the TLD crystals to be "dumped." The crystals will then be recovered from the dosimeter and "read" on the TLD reader. Through careful study we hope to be able to relate the resulting temperature glow curves to the microwave power incident on the dosimeter.

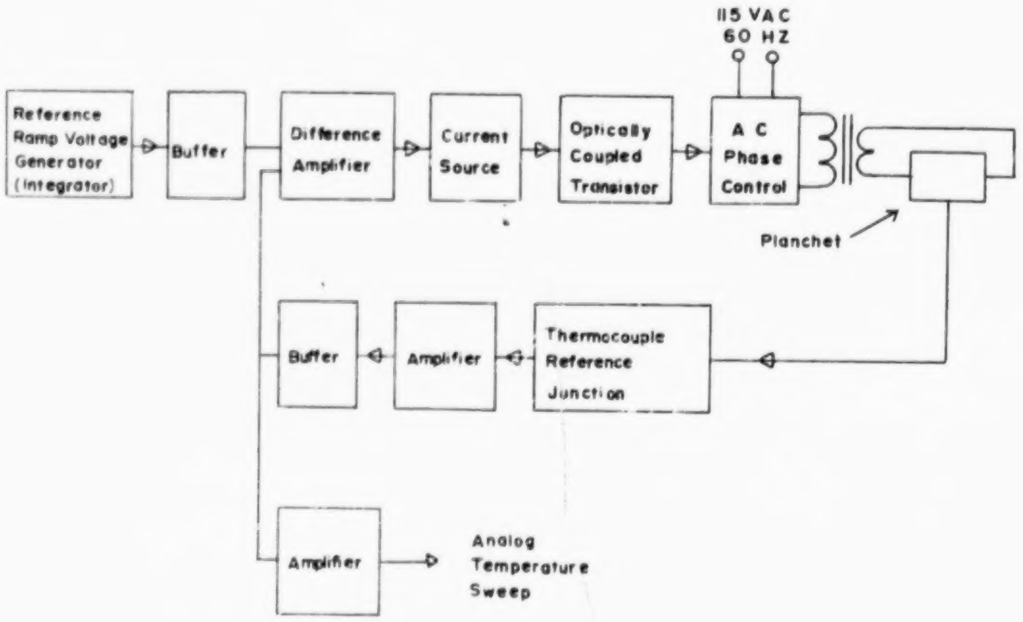
Features of the Temperature Controller

Features of the TLD temperature controller are:

1) The heating rate will be linear
2) The heating rate will be adjustable from 1° C/second to 15° C/second
3) Maximum planchet temperature will be 300° C
4) Any end point temperature may be selected between ambient temperature and 300° C
5) The temperature of the planchet will be controlled and reproducible to within 1° C for all heating rates between 1° C/second and 15° C/second (this corresponds to heating times of 300 seconds to 20 seconds respectively, if the crystals are to be heated to the maximum endpoint temperature of 300° C.)

Description of Circuit

A block diagram of the temperature controller is shown below.


115 VAC
60 HZ
Reference Ramp Voltage Generator (Integrator)
Buffer
Difference Amplifier
Current Source
Optically Coupled Transistor
A C Phase Control
Planchet
Buffer
Amplifier
Thermocouple Reference Junction
Amplifier
Analog Temperature Sweep

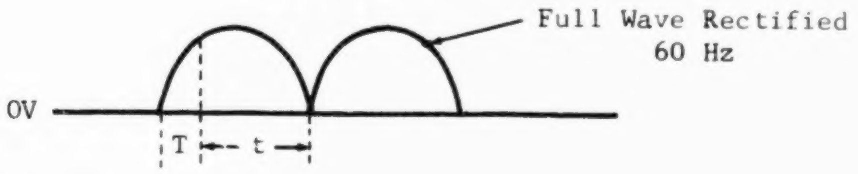
There are several methods available to heat the TLD crystals; however, the method that lends itself to accurate temperature control is heating a metal planchet electrically by passing a large current through it. Power to the planchet can be controlled easily through the use of an "AC phase control"[2] circuit.

The planchet temperature will be monitored with a thermocouple. The thermocouple voltage, which is in the low millivolt range, will be amplified by a low offset-voltage drift operational amplifier. This voltage is then buffered and directed to a difference amplifier.

The reference signal, which the planchet temperature is made to track, is generated in the "Reference Ramp Voltage Generator." This circuit is an integrator which incorporates a field effect transistor (FET) operational amplifier to minimize current drift with changing ambient temperature. The signal that is integrated is simply a DC voltage derived from a temperature-compensated Zener diode. The ramp voltage is buffered and compared with the voltage representing the planchet temperature. The difference amplifier generates an error voltage proportional to the difference between the amplified thermocouple voltage and the reference voltage.

In order to electrically isolate these portions of the circuit from the 60 Hz power circuitry, an optically coupled transistor is used. The optically coupled transistor acts as a variable resistor in the AC phase control circuit.

The phase control circuit determines how long (T) the application of power to the step-down transformer will be delayed after a zero crossing of the line voltage (see sketch below). The transformer is powered for the time represented by t. This phase control effectively controls the electrical power to the planchet.



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[2] General Electric SCR Manual (4th ed), Chapter 9, AC Phase Control. General Electric Company, Chicago, 1967.

D. TINEA CAPITIS DOSIMETRY[1]

Wah Lee, Harry D. Youmans

Tinea capitis (ringworm of the scalp) is a fungal scalp infection that was prevalent among prepubescent children during the 1940's and 50's. Patients with this skin disorder were routinely treated with X-ray therapy before griseofulvin became the treatment of choice in 1958. The method of X-ray therapy commonly used was the Adamson-Kienbock five field treatment technique (Figure 68). This method requires the administration of five radiation fields of 300-500 R per field evenly distributed on the entire scalp. The radiation beam qualities commonly employed for treatment varied from 70-100 kVp with HVT's of about 0.6-1.5 mm Al. Cipollaro, et al., had estimated that as many as 200,000 children worldwide had received this form of radiation therapy since the early 1900's.[2] A recent epidemiological study of tinea capitis by Albert and Omran indicated that the irradiated patients suffered a substantial increase in mental disorder, cancer, and permanent partial alopecia when compared to the non-irradiated controls.[3] A study conducted in this laboratory recently has shown that the lipid composition of beagle pup brain irradiated in vivo was also altered by radiation doses comparable to those received by children irradiated for the treatment of tinea capitis. As a result of the above findings, the Epidemiologic Studies Branch is currently collaborating with the Israeli government to study the latent consequence of 20,000 children irradiated in Israel for the treatment of tinea capitis during the 1940's and 50's. The objective of our laboratory study is to determine retrospectively the doses to the brain, eye, and thyroid of those children treated for tinea capitis with X-radiation therapy.

Dose Estimation

The method developed in this laboratory for estimating doses to the brain, eye, and thyroid of children irradiated by the most common X-ray treatment techniques for tinea capitis are summarized in this section.

Brain Dose Estimation

A complete brain dose distribution in a 3-12-year-old child can be estimated to a fair degree of accuracy for a given set of conditions of irradiation using Figures 69 and 70. Figure 69 shows the relationship of the mean brain dose as function of kVp and HVT. The arbitrary unit is defined as the product of kVp and HVT (in mm Al). The necessity of introducing the arbitrary unit in Figure 69 is to account for the dose dependence of both kVp and HVT under a common variable. The employment

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[1] The preliminary results of this study were published in the 1968 Summary Report. The study was completed in 1969, and the final results were presented before the Second International Conference on Medical Physics in Boston, Massachusetts, on August 14, 1969. The full manuscript of this paper has been completed and is currently being reviewed.

[2] Cipollaro, A.C. et al. New York J. Med. 59:3033, 1959.

[3] Albert, R.E. and Omran, A.R. Arch. Environ. Health 10:899, 1968.

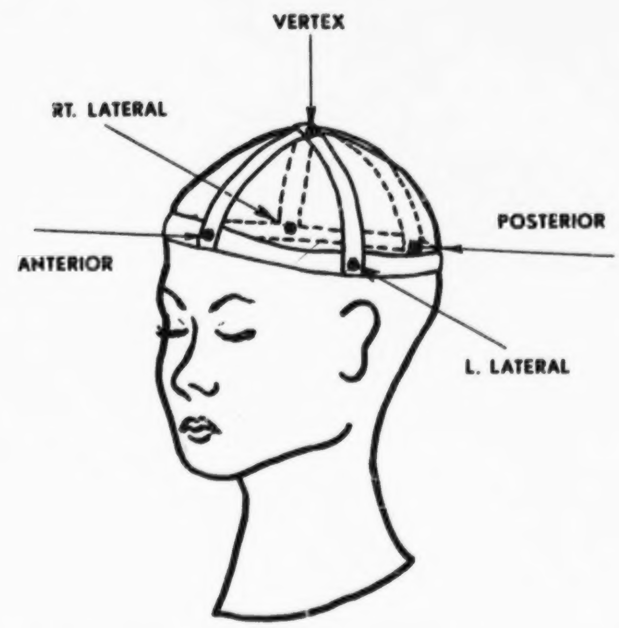
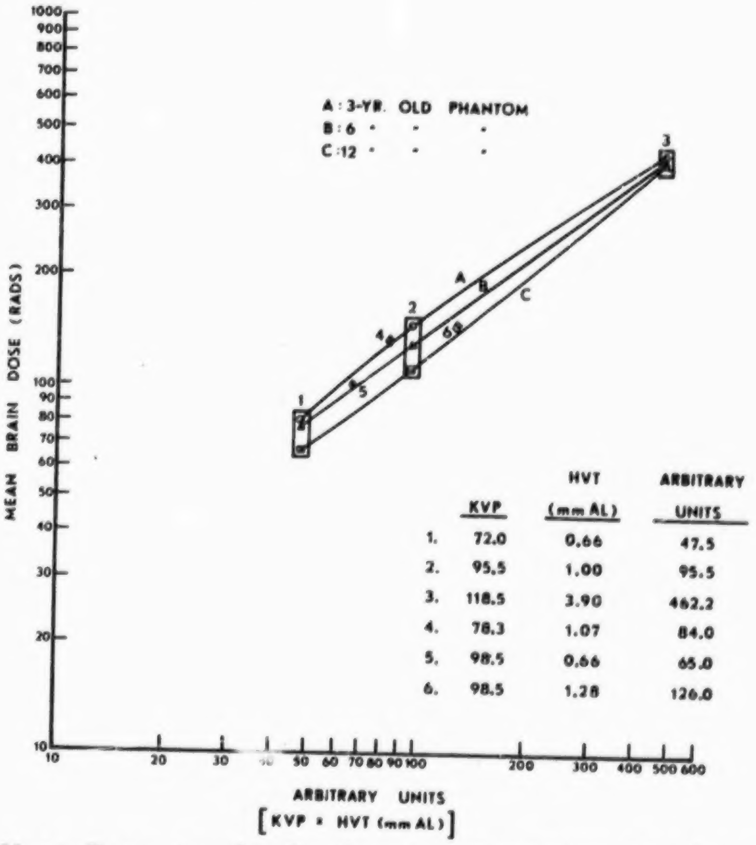


Figure 68. Adamson-Kienböck Five-Field Treatment Technique. The five-field centering points are equal distance from their neighboring points. The five points are usually marked on the scalp of the patient with the aid of a treatment "cap."



	KVP	HVT (mm AL)	ARBITRARY UNITS
1.	72.0	0.66	47.5
2.	95.5	1.00	95.5
3.	118.5	3.90	462.2
4.	78.3	1.07	84.0
5.	98.5	0.66	65.0
6.	98.5	1.28	126.0

Figure 69. Mean Dose to the Brain as a Function of kVp and HVT. The arbitrary unit is defined as the product of kVp and HVT (in mm Al). The numbers on the curves refer to the X-ray technique as described at the bottom of the Figure.

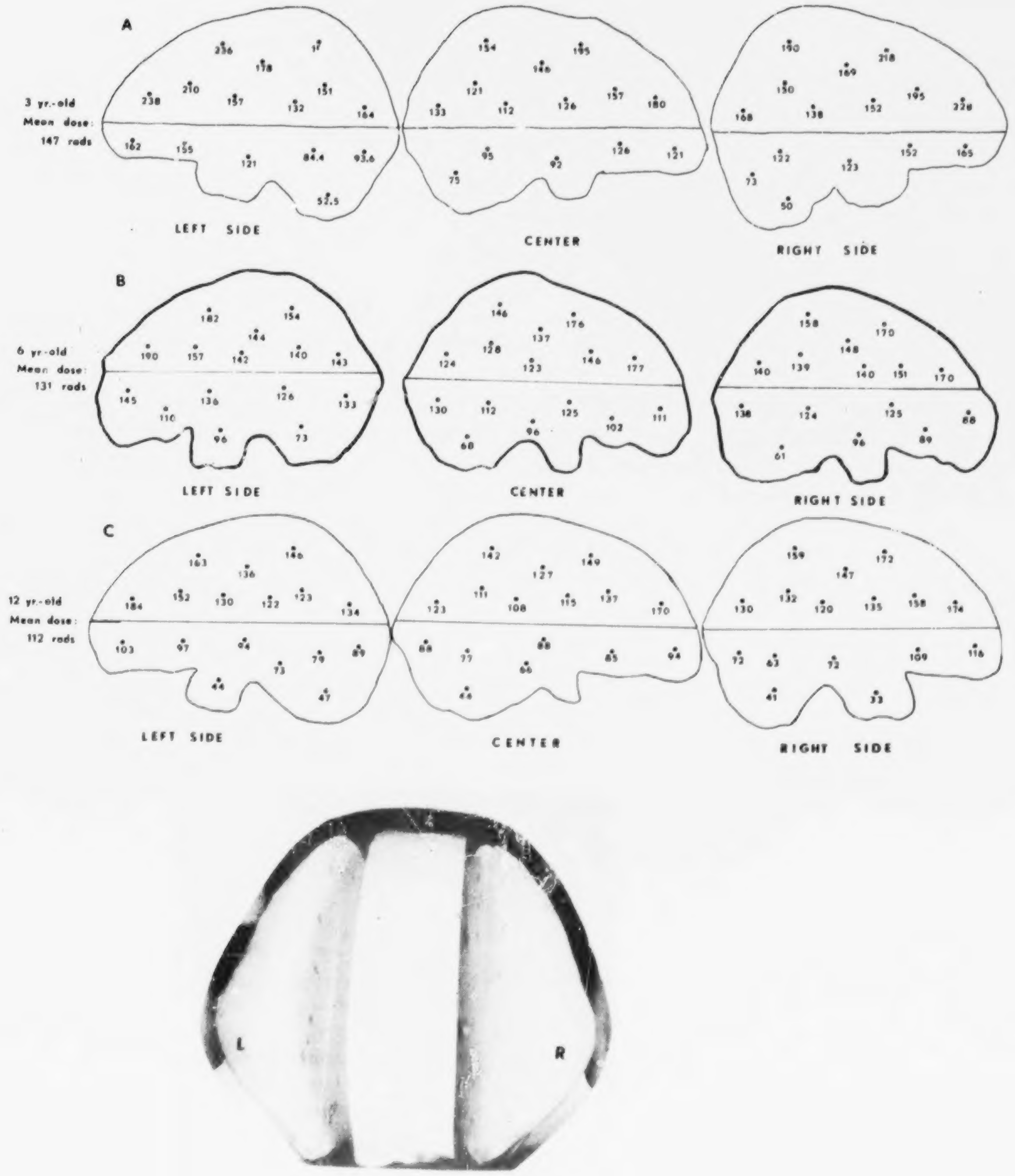

A
3 yr.-old
Mean dose:
147 rads
236
17
178
210
238
157
132
151
164
162
155
121
84.4
93.6
52.5
LEFT SIDE
154
195
148
121
133
112
126
157
180
95
92
126
121
75
CENTER
190
218
169
150
168
138
152
195
228
122
123
152
165
73
50
RIGHT SIDE
B
6 yr-old
Mean dose:
131 rads
182
154
144
190
157
142
140
143
145
110
136
126
133
96
73
LEFT SIDE
146
176
137
128
124
123
146
177
130
112
125
102
111
96
68
CENTER
158
170
148
140
139
140
151
170
138
124
125
88
96
89
61
RIGHT SIDE
C
12 yr.-old
Mean dose:
112 rads
163
146
136
184
152
130
122
123
134
103
97
94
73
79
89
44
47
LEFT SIDE
142
149
127
111
123
108
115
137
170
88
77
88
66
85
94
44
CENTER
159
172
147
130
132
120
135
158
174
72
63
72
109
116
41
33
RIGHT SIDE
L
R

Figure 70. Dose Distribution in the 3-, 6-, and 12-Year-Old Brain Phantoms. The numbers in the drawings are in rads. Note the dose symmetry along the vertical axis in the brain phantoms. Irradiation technique: 95.5 kVp, 1.00 mm Al HVT (95.5 Arbitrary Units).

of the arbitrary unit enables one to estimate any brain dose distribution from various X-ray treatment techniques due to different combinations of kVp and HVT commonly employed in patient irradiation.

Basically, three beams: numbers 1, 2, and 3 (Figure 69) were used to irradiate all three phantoms to estimate the functional relationship of the mean brain dose and the arbitrary units. Numbers 4, 5, and 6 are the test points for the three functions under extreme combinations of kVp and HVT commonly used in patient therapy. The functions still hold with only 5 percent of deviation from the measured values on the average.

Once the mean brain dose is established from Figure 69, one can estimate the dose to almost anywhere in the brain with the aid of Figure 70 This estimation is possible because the ratio of any two mean brain doses is approximately equal to the ratio of their respective doses at any given dosimeter location in a given phantom for any two irradiating beams employed in this study. For example, the mean brain dose in Figure 70A is 147 rads when the 3-year-old phantom was irradiated with a beam of 95.5 kVp, 1.00 mm AL HVT (95.5 arb. units). When the 3-year-old phantom was irradiated with the 72 kVp, 0.66 mm Al HVT (47.5 arb. units) beam, a mean brain dose of 80.1 rads was obtained. The ratio of these two mean brain doses is 80.1/147, which approximately equals the ratio of their respective doses at any given dosimeter location in Figure 70A Therefore, once a mean brain dose is established from Figure 69[4] one can choose an appropriate dose distribution[5] in Figure 70 for calculating the new dose distribution with the following equation:

---

[4] Linear interpolation of doses can be applied to the intervals of the 3-6-year-old and the 6-12-year-old groups. Differences in exposure per field and treatment distance (SSD), from those used in this study (400 R per field, 22 cm SSD) can be adjusted to our irradiating conditions by multiplying the mean brain dose obtained in Figure 69 by the following correction factors:

1. Correction factor for different exposure per field =

   $\frac{X}{400}$, where X is the number of roentgens per field used in patient therapy.

2. Correction factor for different SSD = $\frac{1}{2}(1 + F^2)$

where: $F = \frac{f_x}{f_s} \frac{(f_s + d)}{(f_x + d)}$

f_s = the treatment distance used in this study (22 cm)
f_x = any treatment distance commonly used in patient therapy
d = the depth in cm
(For all practical purposes, d can be assumed to be 5 cm for brain, 10 cm for eye and pituitary, and 20 cm for thyroid.)

Correction factor for SSD in the range of 20-25 cm can be neglected if accuracy of dose smaller than 3 percent is not significant.

$$\frac{D_x}{D_s} = \frac{d_x}{d_s} \quad \text{or} \quad d_x = \frac{D_x}{D_s} d_s$$

where:

D_x = the mean brain dose of an unknown brain dose distribution,
D_s = a mean brain dose of a known brain dose distribution in Figure 70
d_s = a known dose value anywhere in the known brain dose distribution in Figure 70 , and
d_x = the unknown dose at the same dosimeter location as d_s.

This method of estimating dose distribution in the brain from the mean brain doses seems valid in all the phantoms between any two beam qualities employed in this study. It is particularly effective when the range of radiation qualities is limited to 72-98.5 kVp with HVT's of 0.66-1.28 mm Al (the most common techniques employed in patient therapy). Within this limited range of radiation qualities, the estimated doses deviate from the measured values by an average standard deviation of only 4.8 percent.

Eye, Thyroid, and Pituitary Doses

Doses to the eye, thyroid, and the pituitary are shown in Figure 71 These organs receive their doses mainly from scattered radiation at large depths. Consequently, large uncertainties are introduced in their measurements and estimations. Estimated standard deviations of doses to the eye, pituitary, and thyroid are 6 percent, 9 percent, and 11 percent of the mean values respectively. In Figure 71 the thyroid doses in the 12-year-old phantom appear to be higher than those of the 6-year-old one. This discrepancy is due to the longer neck of the 6-year-old phantom.

The pituitary doses of the 6-year-old phantom were not measured. However, because of similarities in cranium thickness, brain size (Table 28) and eye doses (Figure 71), doses to the pituitary in the 6-year-old phantom should be very similar to those of the 12-year-old one. Despite the shortcomings in estimating doses to the eye, thyroid, and the pituitary, Figure 71 should provide a reasonable range of doses to these organs resulting from tinea capitis therapy.

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[5] Figure 70A can be used for the ages of 3-4, 70B for ages 5-8, and 70C for ages 9-12.

TABLE 28. DIMENSIONS OF PHANTOMS, AGES 3, 6, AND 12 YEARS

Phantom	Max. Dia., cm		Cranium Thickness, cm		Mean Scalp Thickness	Brain
Age, yr	AP	Lat	Mean	Range	(Mix-D), mm	Mass,[a] g
3	15.5	12.0	2.4	1.5 - 3.0	4 ± 1	1055
6	16.0	12.5	2.9	2.5 - 4.0	4 ± 1	1136
12	16.5	13.0	3.3	2.5 - 4.0	4 ± 1	1140

a. The mass of the brains in the phantoms was estimated by measuring volume of the skull cavities in the phantoms.

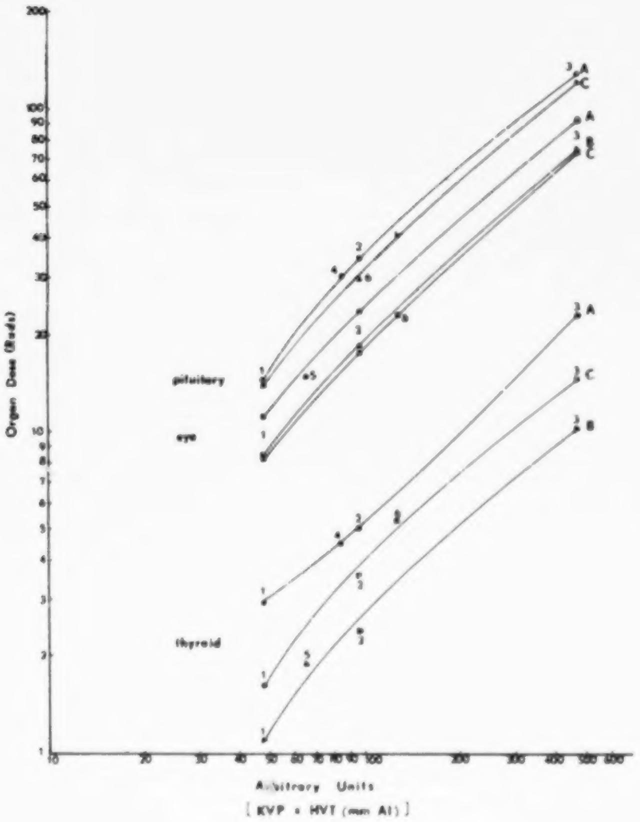

Figure 71. Doses to the Eye, the Thyroid, and the Pituitary as Functions of kVp and HVT. The arbitrary unit is defined as the product of kVp and HVT (in mm Al). Curves A, B, and C represent the 3-, 6-, and 12-year-old phantoms respectively. The numbers on the curves refer to the X-ray techniques as described in Figure 69.

X-Ray Tube Potential Calibration

The peak X-ray tube potential (kVp) of the X-ray machine used in this study was calibrated with a spectrometer (Victoreen SCIPP 1600) coupled to a Ge(Li) solid state detector cooled with liquid nitrogen. The energy scale of the spectrometer was calibrated against monoenergetic photons from the emissions of radioisotopes at four energy levels: 47 keV (^{210}Pb), 14.4 keV and 121.6 keV (^{57}Co), and 88.0 keV (^{109}Cd). The energy scale was found to be linear within the above range of photon energies. A calibrating spectrum with ^{210}Pb is presented in Figure 72. The 47.0 keV spectrum of ^{210}Pb resembles a line with its peak height at channel 75 and has no channel containing counts above the base line level beyond channel 76. The highest channel containing counts above the base-line level in a given spectrum corresponds to the maximum energy of that spectrum. Therefore, when the relationship of a series of known monoenergetic photon spectra and their corresponding highest channel containing counts above the baseline level are known, the maximum energy of an unknown photon spectrum can be determined by its highest channel registered in the spectrometer. Figure 73 is a 57 kVp X-ray spectrum taken with an expanded scale spectrometer display. This technique brings out a distinct separation of the base-line channels from those of the spectrum. The highest channel containing counts above the base-line level in a given X-ray spectrum was observed to shift no more than one channel within the count rate of 300-3000 counts per minute (the count rates commonly employed in our spectral measurements) and for a total count up to 50,000. One channel in Figure 73 corresponds to 0.6 keV, which is the approximate uncertainty for this method of calibrating the peak X-ray tube potentials (kVp) in this study.

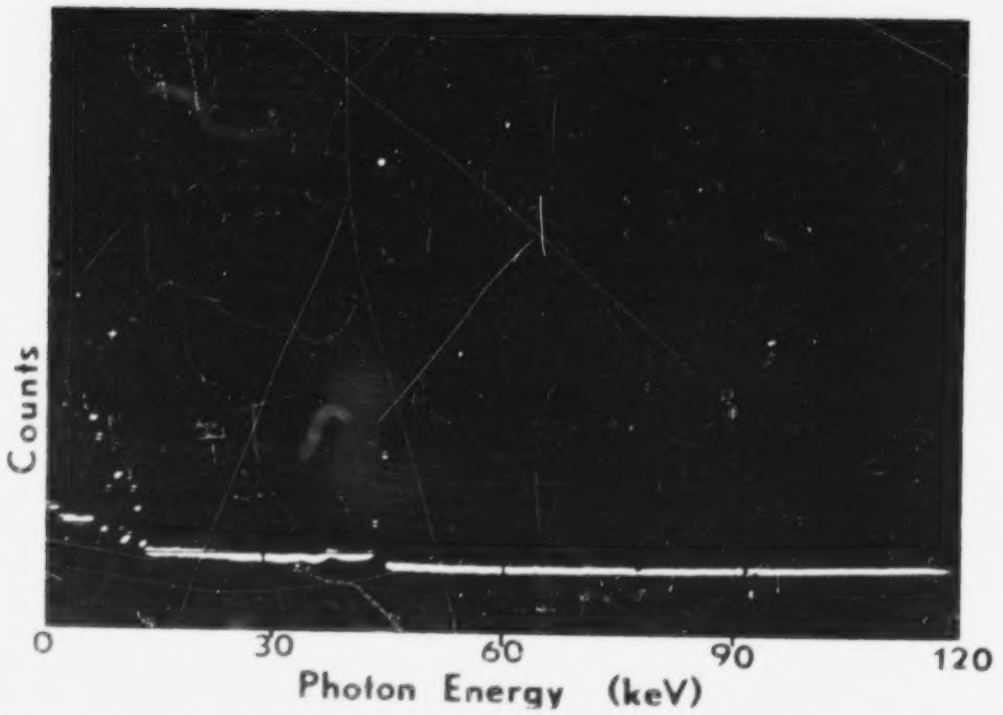

Figure 72. A 47 keV Monoenergetic Photon Spectrum from the Emission of ^{210}Pb. Scale of calibration: 0.6 keV/channel, detector Ge(Li) cooled with liquid nitrogen.

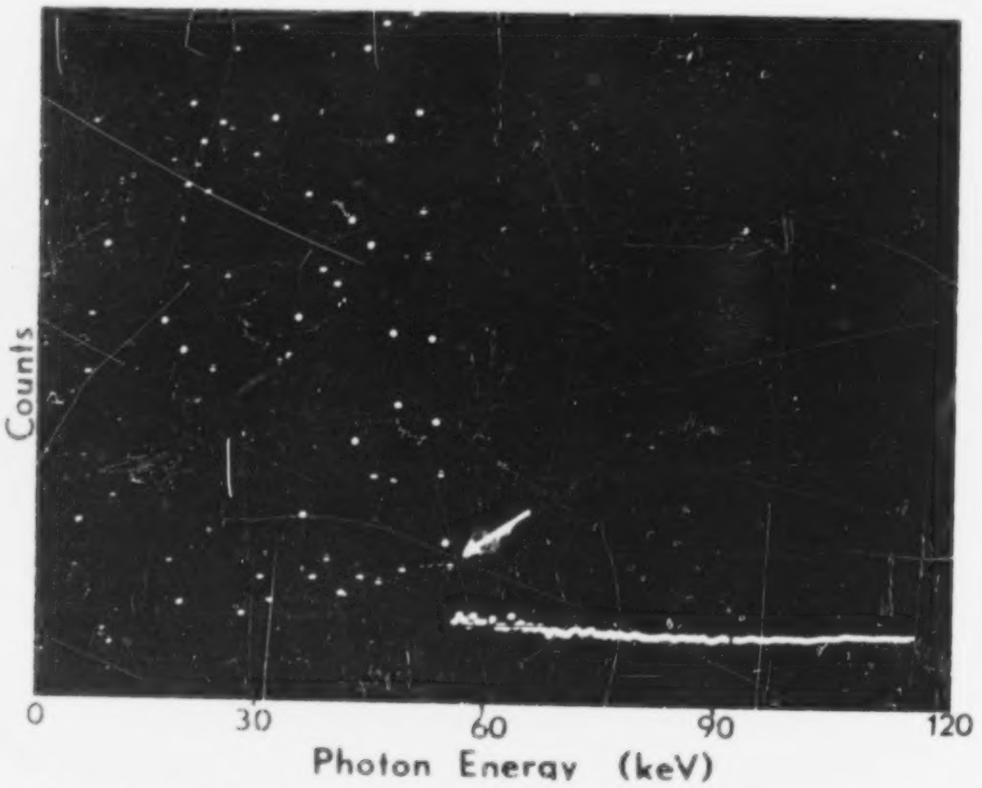

Figure 73. A 57 kVp X-Ray Spectrum Taken With an Expanded Scale Spectrometer Display which Makes a Distinct Separation of the Base Line Channels from Those of the Spectrum. The maximum photon energy of the spectrum corresponding to the maximum potential across the X-ray tube (kVp) is determined by observing the highest channel containing counts above the base line as indicated by the arrow.

E. IONIZING RADIATION DOSIMETRY AND IRRADIATION SERVICE

Wah Lee, James W. Rolofson, Lynda D. Kramer

Irradiation Service

One of the functions of the Dosimetric Study Section is to provide irradiation services and dosimetric support for the Division of Biological Effects. Irradiation sources available in the Dosimetric Studies Section are: a 1,000 curie ^{137}Cs radioisotope unit, a 250 kVp therapeutic X-ray generator, a 120 kVp superficial therapeutic X-ray unit, and a 0-75 kVp very low energy, high intensity X-ray generator.

From January 1, 1969 to December 1, 1969, the Dosimetric Studies Section used these facilities to irradiate 2,350 hamsters, 1,287 rats, 335 mice, 48 swine, and 552 cell culture specimens.[1]

Color TV Radiation Dosimetry

Ever since a color TV manufacturer informed the Public Health Service that some of their color television receivers were emitting radiation in excess of the guide line set by the NCRP, the Division of Biological Effects has been investigating the possible biological effect of color TV irradiation. Typical radiation spectra from the emissions of color television receivers as reported by Wang and Hersh in the Conference on Detection and Measurement of X-Radiation from Color Television Receivers were cross-checked in our laboratory. Consequently, a radiation spectrum was generated to approximately match the composite color TV radiation spectra with the high intensity, low energy X-ray machine at 30 kVcp, and 3.09 mm aluminum added filtration (Figure 74). The mock color TV spectrum has an HVT of 1.1 mm aluminum and an output of 64 R per minute at 30.0 cm TSD.

Most investigators wanted to compare the biological effectiveness in animals and cell cultures from color TV-like radiation with the conventional medium energy X ray (250 kVp). To validate these comparisons, dose distribution as well as the total dose from both radiation sources were to be compared in the biological specimen under consideration. Dose distributions in animals such as mouse and in the eyes of swine were measured with LiF micro-dosimeters in animal cadavers and Mix-D phantoms. Discrepancies in dose distribution from the two radiation sources were approximately compensated by adjusting the target-to-source distance (TSD) and/or the amount of added filtration to one or both beams.

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[1] These numbers exclude the numbers of biological specimens shammed with the radiation facilities. Procedures for shamming biological specimens are identical to those irradiated except with the radiation source off.

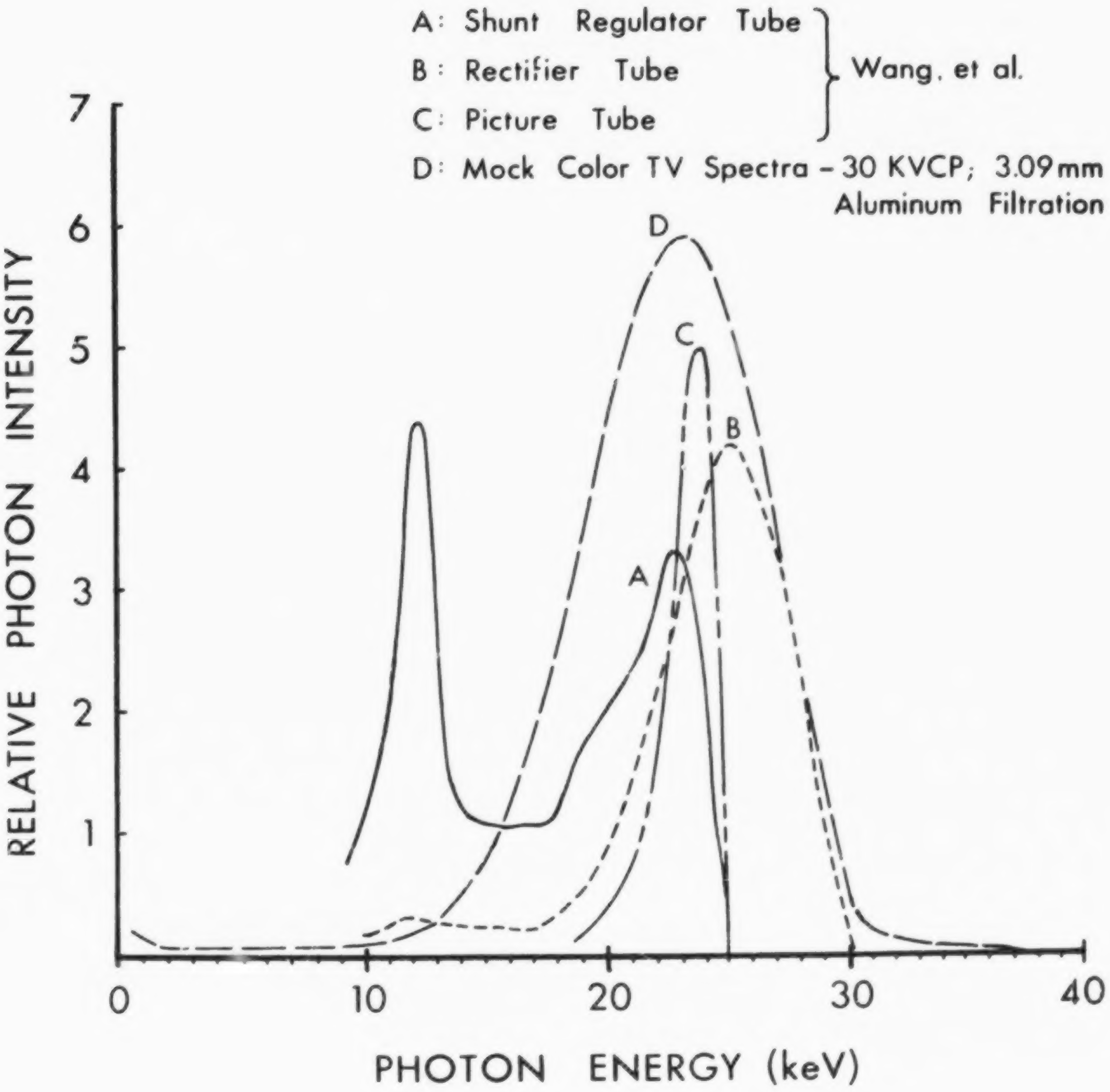

Figure 74. Comparison of X-Ray Emission Spectra from Various Color TV Receiver Components and Mock Spectra Used for Irradiation of Mice. Curves A, B, and C were obtained from Wang et al., Spectral and Spectral Distribution of X Rays from Color Television Receivers, p. 53-72, In Conference on Detection and Measurement of X-Radiation from Color Television Receivers, U.S. Department of Health, Education, and Welfare and Electronic Industries Association, Washington, D.C., March 1968.

Age Sensitivity of the Chinese Hamster

The Dosimetric Studies Section participated in another study aimed at evaluating the sensitivity of Chinese hamsters to whole-body radiation at different ages. The hamsters were divided into seven groups from the age of one day to adult. A preliminary study indicated that the differences in the LD_{50} doses among the seven groups of hamsters were quite small. Therefore, to validate the intercomparison among the groups of hamsters, the absorbed dose in each age group had to be determined within ± 8 percent. This task was accomplished with a large dosimetric study utilizing LiF micro-dosimeters in animal cadavers of different age groups. Dose distributions and dose rates were also carefully determined among the seven groups of hamsters.

Dose Rate Effect

The Dosimetric Studies Section also contributed to the study of the biological effect on Chinese hamsters of a given radiation dose from two widely different sources of radiation and at different dose rates. One radiation source was a 4 MV pulse X-ray generator[2] with a dose rate of approximately 10^{11} rads/min; the other was a conventional, medium-energy X-ray generator with a dose rate of 50 rads/min. LiF dosimeters were chosen for this study because of their dose rate independence up to about 10^{12} rads per minute. Discrepancy in energy response for the LiF dosimeters from the two sources of radiation was adjusted by calibrating the dosimeters from the emissions of ^{137}Cs for the 4 MV X-ray spectrum, and the other, the radiating spectrum itself. As usual, precautions were taken to insure that the dose distribution and the total dose to the animal from each radiation source were comparable.

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[2] This radiation facility was provided by the Naval Ordinance Laboratory with the collaboration of its laboratory staff.

GENETIC STUDIES SECTION

Dr. William M. Leach, Chief

Effects of the various radiations of the electromagnetic spectrum on the genetic material, the genetic apparatus, and inheritance have comprised the research interests of the Genetic Studies Section during 1969. The Section includes the researchers whose interests were oriented toward morphological research with cells in tissue culture prior to the assumption of responsibilities under Public Law 90-602, the Radiation Control for Health and Safety Act of 1968. Some of the studies of cell morphology have been continued.

The continued development of the research program is expected to yield more concrete and complete results than are reported in this annual summary. The summary of the Section's accomplishments during 1969 provides both a concise public statement of use of public monies toward the eventual improvement of public health and the human environment, and an opportunity for the personnel of the Section to review again their individual accomplishments in the larger context of program goals.

Some studies on the modification of inheritance by radiation have been initiated. Special interests and technical talents have developed some novel potential approaches to problems in assessing radiation effects on inheritance.

A. HUMAN STUDIES

Chromosomal Analysis of Persons with Body Burdens of Mixed Radioisotopes

E. C. Beyer, B. Shleien, L. J. Gillespie

A group of individuals occupationally exposed to mixed radionuclides has been analyzed with respect to their body burdens of gamma emitters and to chromosome aberrations. Radioassay was performed at the Northeastern Radiological Health Laboratory with whole-body counting and spectrometric techniques. Relative amounts of gamma emitters detected have been reported elsewhere.[1]

For chromosomal analysis, leukocytes were cultured from peripheral blood. Cells were fixed after 48 hours of phytohemagglutinin-stimulated growth at 38° C. Figure 75 shows photomicrographs of cells with aberrant chromosomes. A total of 100 cells were analyzed from each volunteer. Table 29 shows the results of the analysis.

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[1] T.W. Athey and B. Shleien. Whole-Body Counting of Some Occupationally Exposed Radiation Workers. Northeastern Radiological Health Laboratory, Winchester, Massachusetts, NERHL-69-3, 1969.

TABLE 29. ABERRATIONS IN BLOOD CULTURES FROM RADIONUCLIDE WORKERS[a]

Body Burden, μCi[b]	Cumulative Rem During Employment[c]	Chromated Aberrations, Percent	Chromosome Aberrations, Percent		Aneuploidy
			Stable	Unstable	
0.73	19.7	5	1	1	4
0.63	8.4	4	-	-	13
0.62	4.9	2	2	2	8
0.46	7.3	4	7	1	12
0.23	4.7	7	4	2	14
0.14	1.3	4	3	4	4
0.13	3.5	1	-	1	17
0.12	0.7	3	5	1	9
0.08	1.8	11	2	-	11
0.02	5.5	4	4	1	8

a. 100 cells were scored for each volunteer.

b. Determined from whole-body counting techniques by Northeastern Radiological Health Laboratory within 3 months of cell culture.

c. From employee's records of film badge reading.

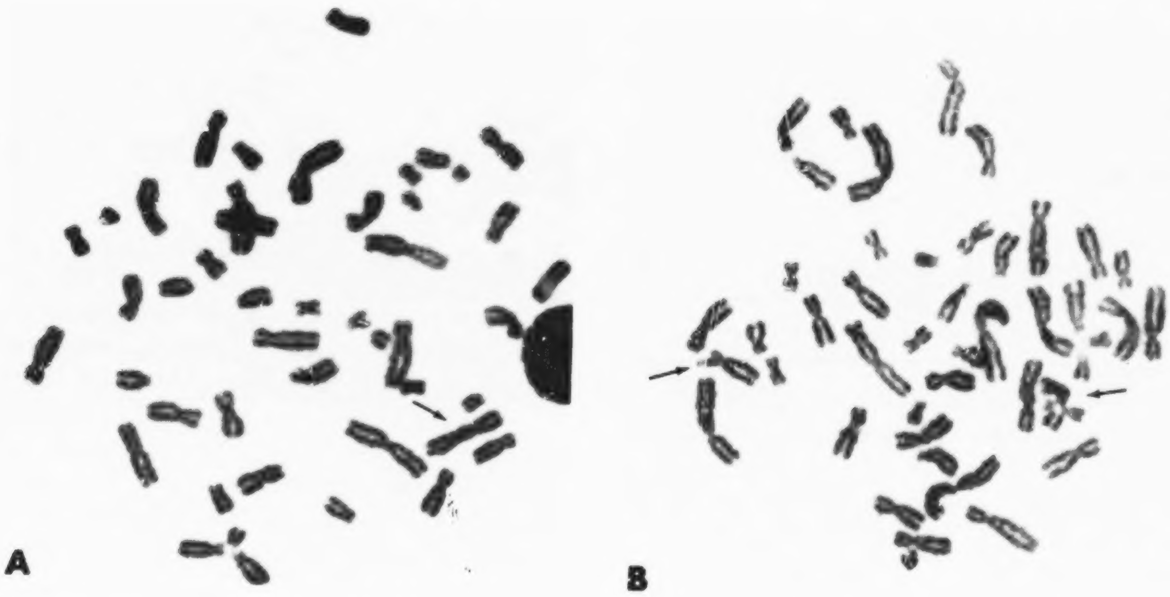

Figure 75. Chromosomal Aberrations in Occupationally Exposed Radionuclide Workers. (A) shows a dicentric chromosome involving chromosomes in the A and C groups. (B) shows a chromatid break and a quadriradial involving chromosomes in the D and E groups.

No clear-cut relationship is apparent between aberration yields and either body burden of gamma emitters or cumulative radiation exposure.

X-Ray Effects on the Nucleolus and on its Role in Chromosome Distribution

S. Brecher

Nucleoli can be caused to persist through mitosis by treating the cells with cobalt. This permits the study of the possible role of nucleoli in the induction of aneuploid cells and the effects of X rays on persistent nucleoli.

Cultures of human peripheral lymphocytes were treated with cobalt, or 100 rads of 250 kVcp X rays, or both, to examine the effects of these agents on nucleolar persistence and on chromosome distribution in Colcemid-blocked metaphase cells. Nucleoli were identified on the basis of azure B bromide staining.

Persistent nucleoli were observed in 57.3 percent of cobalt treated metaphase cells and 39.1 percent of untreated cells. X irradiation did not affect these frequencies. The number of chromosomes attached to persistent nucleoli varied directly with the diameter of the nucleolus. Although cobalt did not affect the direct relationship between chromosome attachment and nucleolar diameter, it appeared to reduce the number of chromosomes attached. The results are summarized in Table 30.

The lack of X-ray effect on nucleolar persistence and the cobalt-induced reduction of attached chromosomes suggest that persistent, essentially intact, nucleoli may not be involved in induction of aneuploidy.

TABLE 30. PROPORTION OF METAPHASE CELLS WITH NUCLEOLI AFTER EXPOSURE TO X RAYS

Cobalt Chloride (g/ml)	X-ray dose							
	0		1		10		100	
	N/T[a]	%	N/T	%	N/T	%	N/T	%
10^{-5}	258/450	57.3[b]	21/50	42.0	19/50	38.0	174/301	57.8[b]
5×10^{-6}	35/100	35.0	17/50	34.0	7/50	14.0		
10^{-6}	119/300	39.7	14/50	28.0	42/100	42.0	35/100	35.0
10^{-7}	88/250	35.2						
10^{-8}	35/100	35.0			18/50	36.0	23/50	46.0
0	352/900	39.1[b]	29/100	29.0	62/200	31.0	236/525	45.0[b]

a. N/T = Total number of metaphase cells with nucleoli over total number of metaphase cells examined.

b. Only these treatment combinations were considered to have a sufficient number of observations for statistical analysis.

The Reversal of Mitotic Effects of Colcemid in Cultures of Human Peripheral Lymphocytes

S. Brecher

To examine the behavior of chromosomes with a "sticky" appearance after acute microwave irradiation at 2450 MHz, a method has been developed to examine cultured lymphocytes in anaphase of mitosis. During anaphase, sister chromatids separate toward opposite poles of the dividing cell. "Sticky" or clumped chromosomes may form chromatin bridges between the two sets of daughter chromosomes.

Cultures of peripheral blood lymphocytes from human volunteers were exposed to Colcemid to arrest mitotic cells at metaphase of mitosis (Figure 76). At the end of exposure to Colcemid, cells were alternately washed with fresh media without Colcemid and concentrated by gentle centrifugation. Cells suspended in fresh medium were incubated at 37-38° C for several hours, then fixed. The frequency of mitotic cells in the cultures varied between 5 and 15 percent. Of the mitotic cells, up to 30 percent were in anaphase (Figure 77) after the reversal of the Colcemid effect.

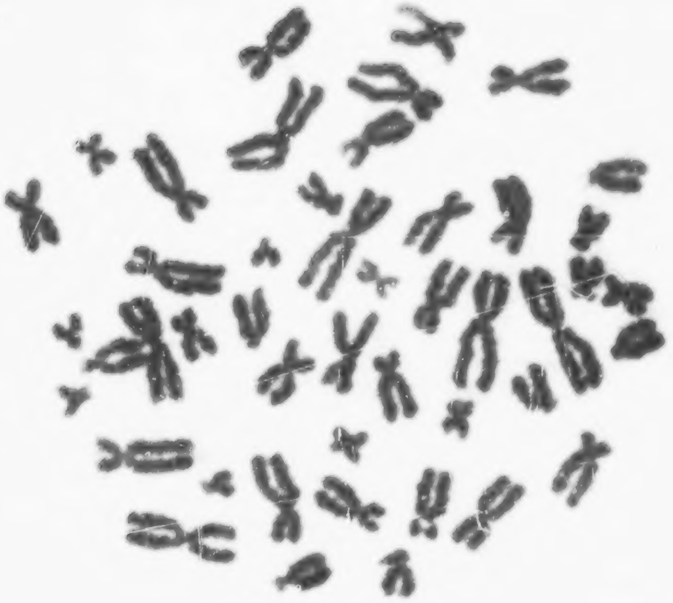

Figure 76. A Colcemid-Blocked Metaphase Cell From a Human Peripheral Lymphocyte Culture.

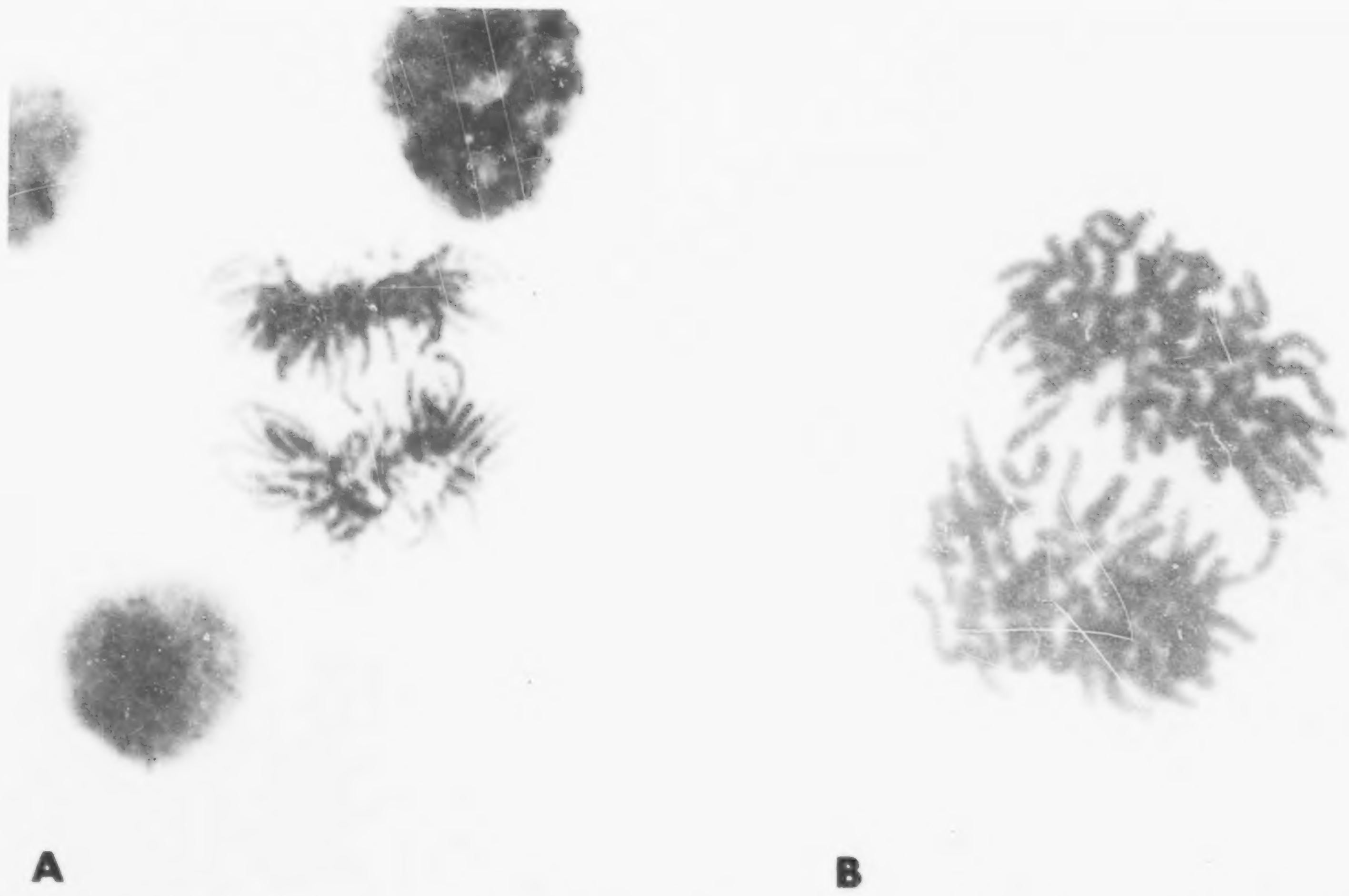

Figure 77. Anaphase Configurations of Cells in Human Peripheral Lymphocyte Cultures After Removal of Medium with Colcemid. (B) shows that chromatid separation has taken place.

Electron Microscopy of Chromosomes from Patients with Aplastic Anemia

J. G. Abuelo

Two patients at the Clinical Center, National Institutes of Health, had aplastic anemia similar to Fanconi's anemia with respect to high incidences of chromosomal abnormalities. In order to examine the aberrant chromosomes with the electron microscope, a collaborative study was initiated with the National Institute of Arthritis and Metabolic Diseases and the National Cancer Institute. The results of the study were published by Hirschman et al.[2]

For electron microscopic observations, chromosomes from peripheral lymphocyte cultures were spread on a Langmuir trough and dried on grids in CO_2 at the critical point.

In the course of electron microscopic examination, breakage was detected as chromatid gaps (distal fragments were not displaced), chromatid breaks (distal fragments were displaced), and acentric fragments. Displaced distal fragments were frequently, but not invariably, connected with the main chromosome mass by one to a few fibers. The diameter of the individual connecting fibers was about 4-5 times below the resolving power of the light microscope.

Other types of aberrations were not observed during electron microscopy. Except for the discontinuities in broken and gapped chromosome arms, the fine structural appearance of the chromosomes was indistinguishable from that of chromosomes from normal volunteers.

Ultrastructure of the X-Irradiated Human Chromosome

J. G. Abuelo, D. E. Moore, S. Brecher, W. M. Leach

The rejoining of strands of chromosomes broken by X irradiation may result in reconstitution of the chromosome so that it appears unaffected, or in rejoining of the ends from other breaks so that a chromosomal rearrangement is observed. In dicentric chromosomal aberrations, the region of rejoining lies between the two centromeres, and it may be possible to identify specifically the sites of rejoining with electron microscopy. The purposes of this study are to determine whether the specific site of rejoining can be detected in dicentric chromosomes and to describe ultrastructural aspects of the region of rejoining.

Cultures of lymphocytes from peripheral blood were prepared from human volunteers and exposed to 300-500 R of 100 kVcp X rays. Dividing cells were accumulated with Colcemid during the last 4 hours of 48 hours of incubation after irradiation. Chromosomes of mitotic cells were spread on the surface of a Langmuir trough, and dried on grids in CO_2 at the critical point.

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[2] Hirschman, R. J. et al. Ann. Internal Med. 71:107-117, 1969.

Chromosomes from irradiated cultures appeared to adhere to each other and did not spread as well as unirradiated chromosomes (Figure 78). Areas of loosely packed chromosomal fibers (arrows in Figure 78B) were observed in preparations of both irradiated and unirradiated cultures, but appeared to be more extensive in irradiated material.

Gaps, chromatid and isochromatid breaks, acentric fragments, rings, translocation and dicentric chromosomes were observed in chromosomes of irradiated cells. Figure 79 shows an apparent chromatid break or gap. Fibers run from the main chromosome mass to the distally displaced portion of the chromatid arm. Figure 80 shows acentric fragments. One of the examples (Figure 80B) appears to be an interstitial "dot" deletion. Figure 81 shows a translocation chromosome. The apparent hiatus in fiber arrangement indicated by an arrow is suspected as the region of rejoining. Similar appearance is seen in dicentric chromosomes (Figure 82) where the region of rejoining must be located between the kinetochores. Adjacent to the disturbed fiber organization is a highly electron opaque region.

The observations of regions of suspected rejoining after X-ray damage suggest that the reunion of broken "ends" of chromosomes does not reestablish the organizational integrity of fibers in the rejoined area.

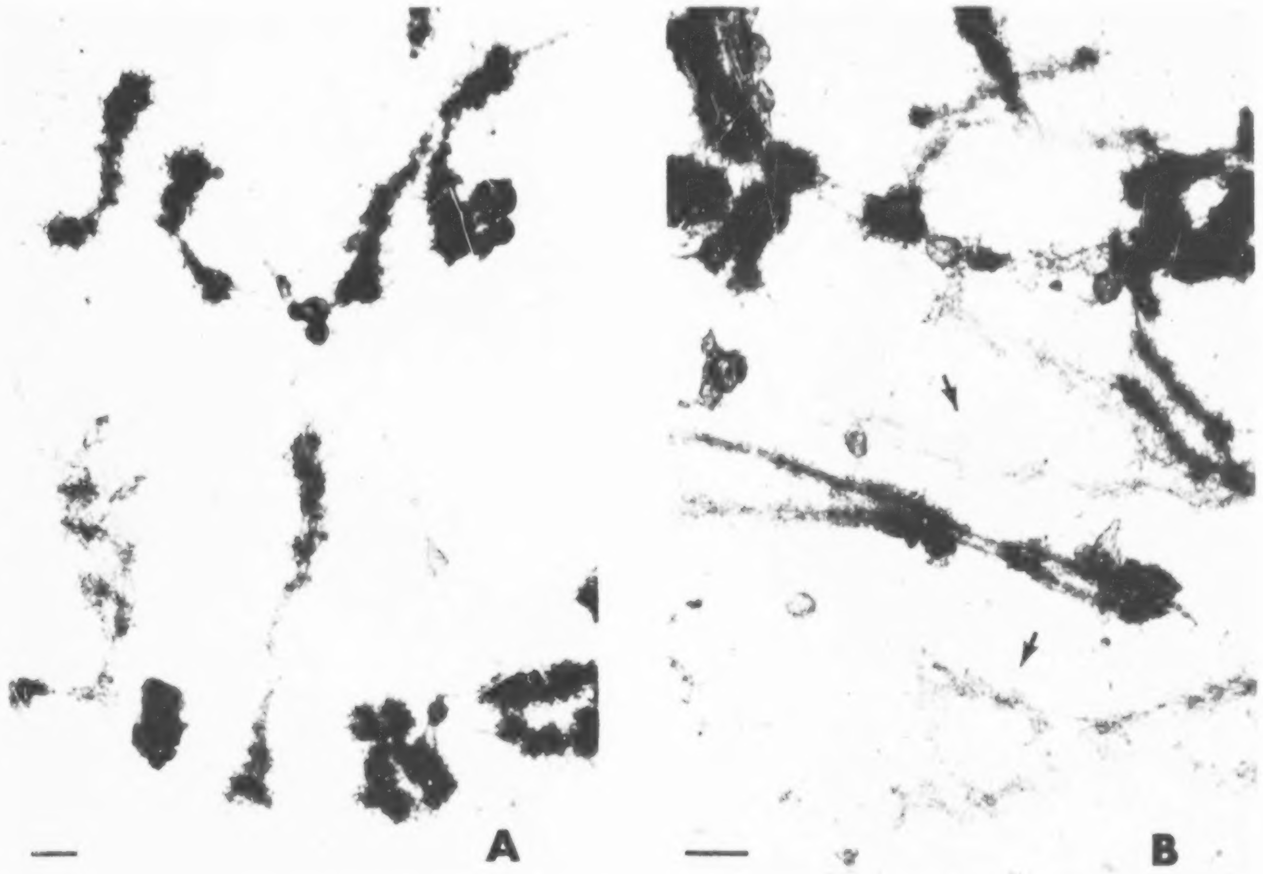

Figure 78. Spread of Whole-Mounted Human Chromosomes. (A) unirradiated; (B) exposed to 300 R. Arrows indicate looser fiber organization of chromosomes. Horizontal bars on this series of electron micrographs represent 1 micron.

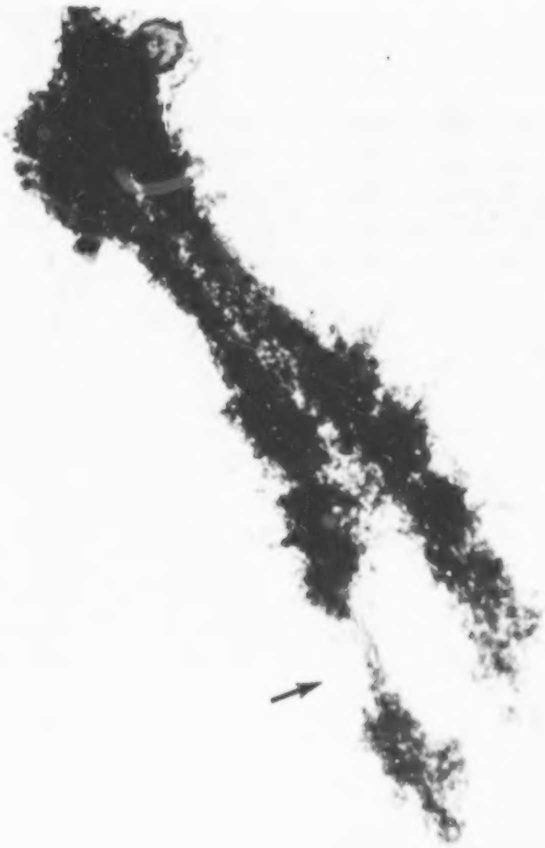

Figure 79. Chromatid Break or Gap in an Irradiated Chromosome. Break region noted by arrow; X-ray exposure, 500 R.

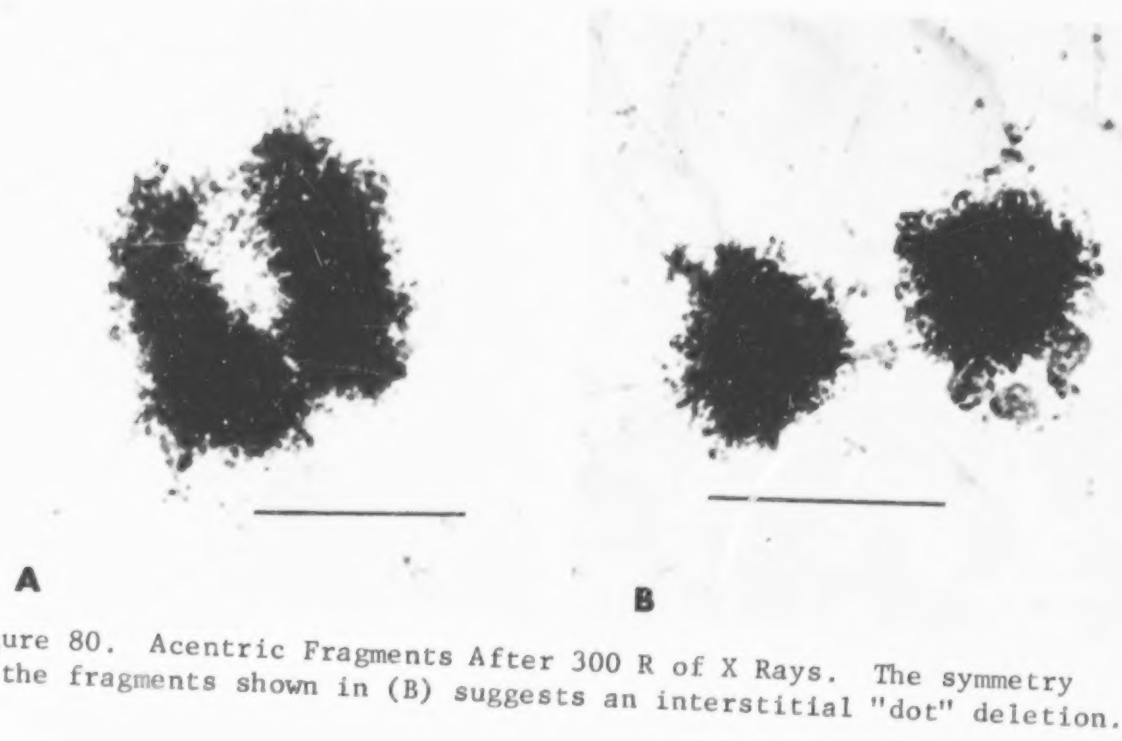

Figure 80. Acentric Fragments After 300 R of X Rays. The symmetry of the fragments shown in (B) suggests an interstitial "dot" deletion.

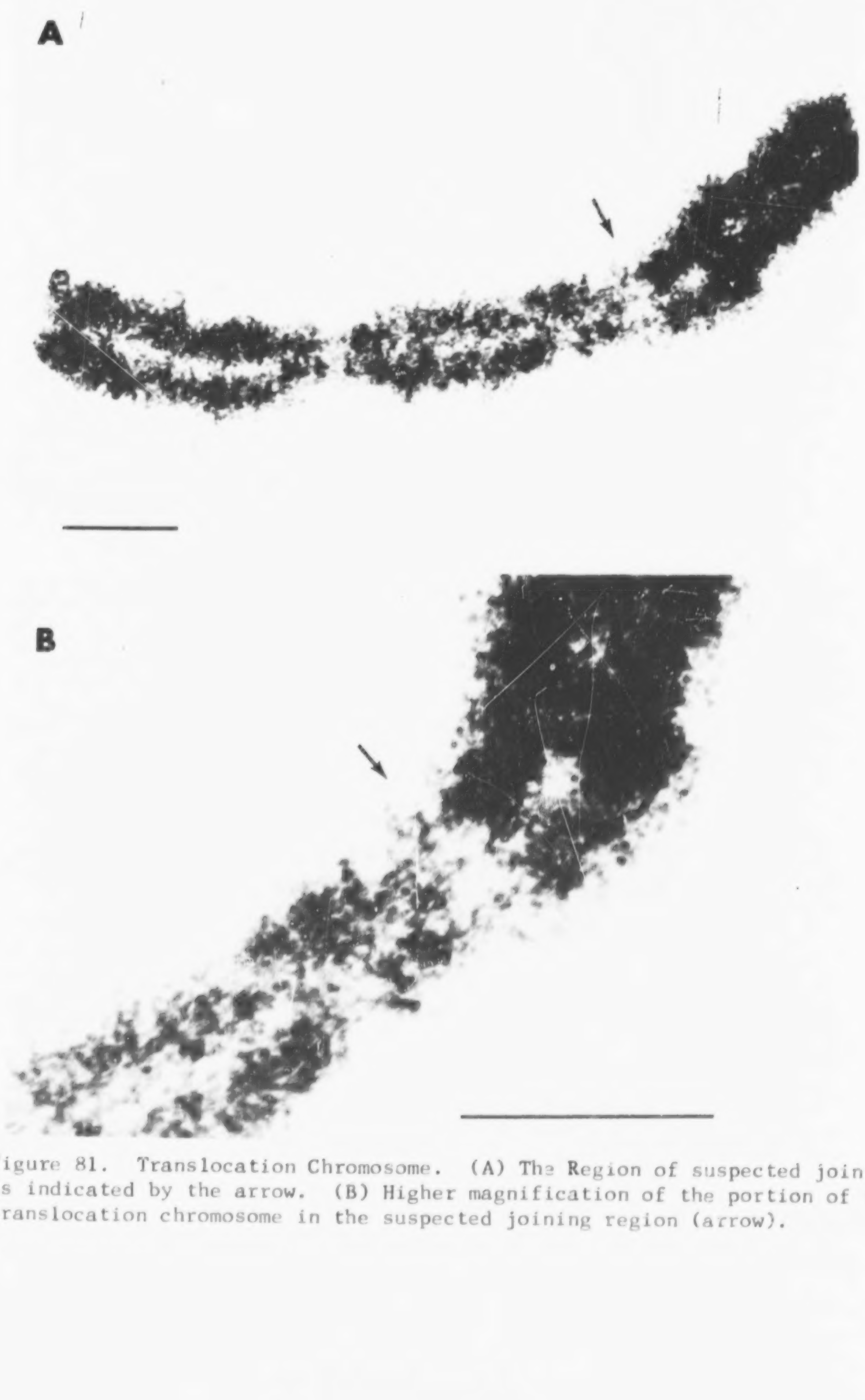

Figure 81. Translocation Chromosome. (A) The Region of suspected joining is indicated by the arrow. (B) Higher magnification of the portion of the translocation chromosome in the suspected joining region (arrow).

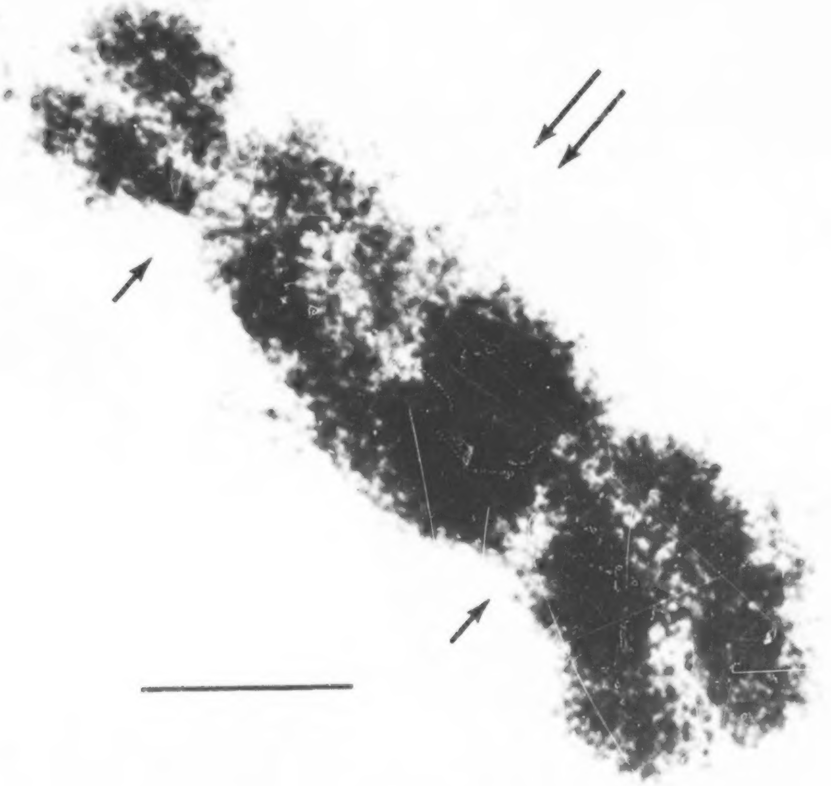

Figure 82. Dicentric Chromosome. Kinetochore regions indicated by single arrows. The joining region in dicentric chromosomes is between kinetochores. Note that the region of suspected joining (double arrows) in the dicentric is similar to the region indicated in Figure 81.

Scanning Electron Microscopy of Human and Chinese Hamster Chromosomes

J. G. Abuelo

The image of a specimen in a scanning electron microscope is formed by electrons scattered from the surface of the specimen. It is possible, therefore, to obtain a visual impression of three dimensions of the specimens. In order to examine the surface of chromosomes, cells from human peripheral lymphocytes or Chinese hamster thyroid cultures were arrested at metaphase with Colcemid and lysed at the surface of a Langmuir trough. Chromosomes were dried on aluminum specimen mounts in CO_2 at the critical point.

Figure 83 shows a partial metaphase spread from Chinese hamster cells. Recognizable chromosomes are outlined for easy identification. Much residual cellular material was seen near most of the chromosome spreads studied.

Figure 84 shows a higher magnification of one of the chromosomes in the previous figure. Thin fibers may be seen between sister chromatid arms. Figure 85 shows a human chromosome with extensive fibers. It is not possible to determine the extent to which the outstretched fibers are artefacts of the technique of lysing cells. The configurations of inter-arm fibers suggest that these fibers are not artefacts of lysing. However, one can only speculate concerning their significance to the bipartite chromosome.

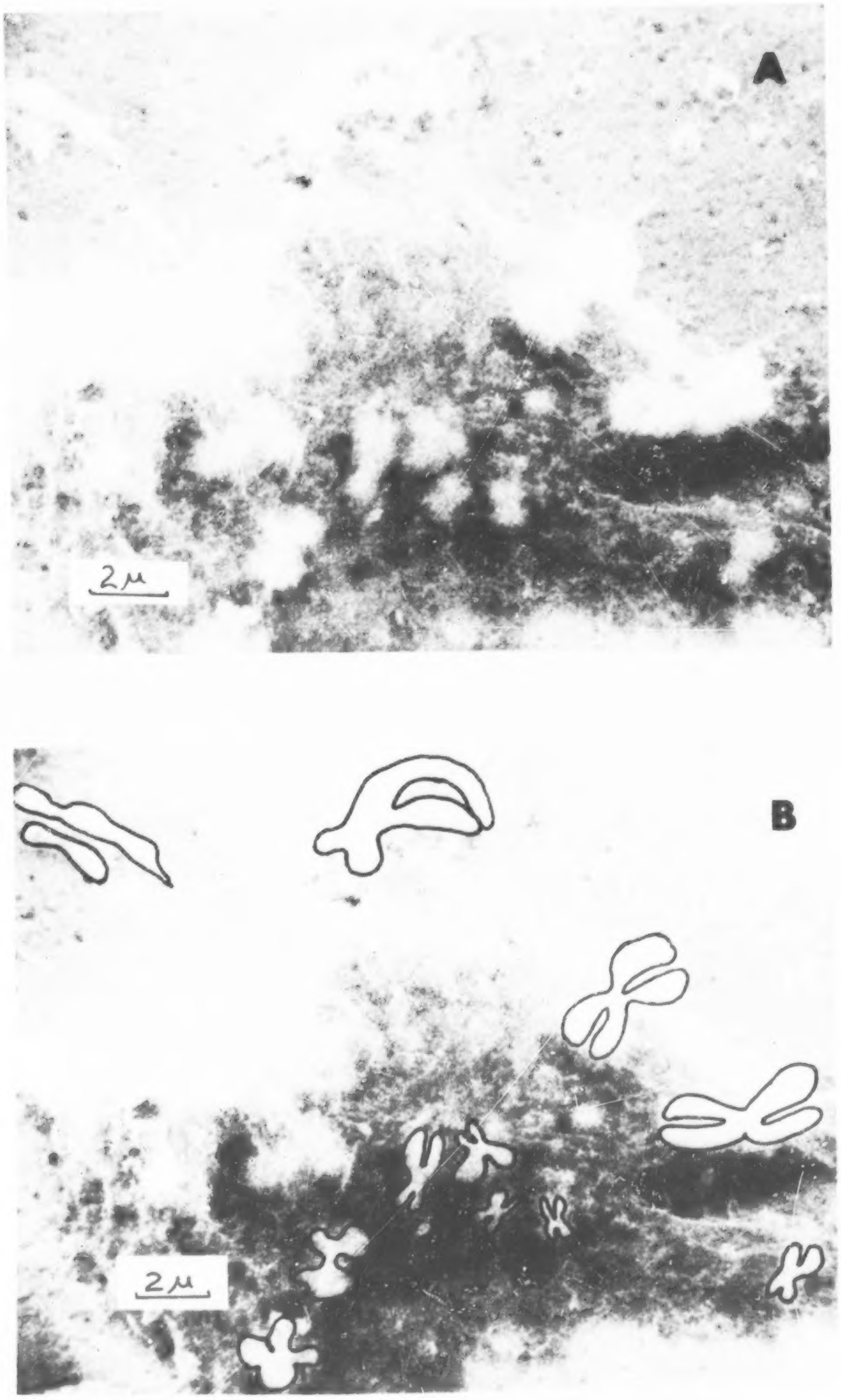

Figure 83. A Spread of Whole-Mounted Chromosomes Viewed with a Scanning Electron Microscope. The recognizable chromosomes are outlined in (B)

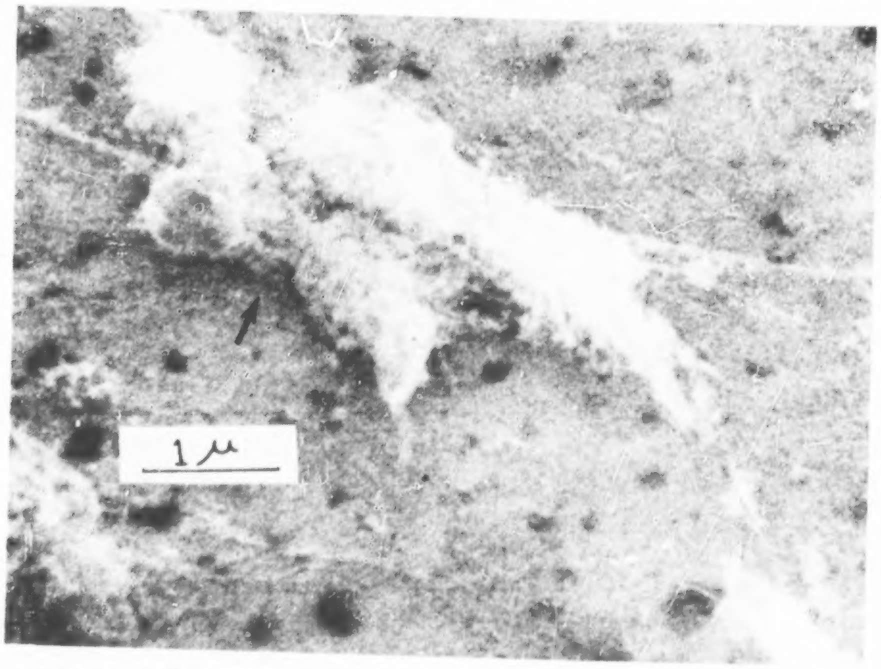

Figure 84. Chinese Hamster Chromosome Shown in Figure 83 (upper left). Note kinetochore region (arrow) and fibers between the long chromatid arms.

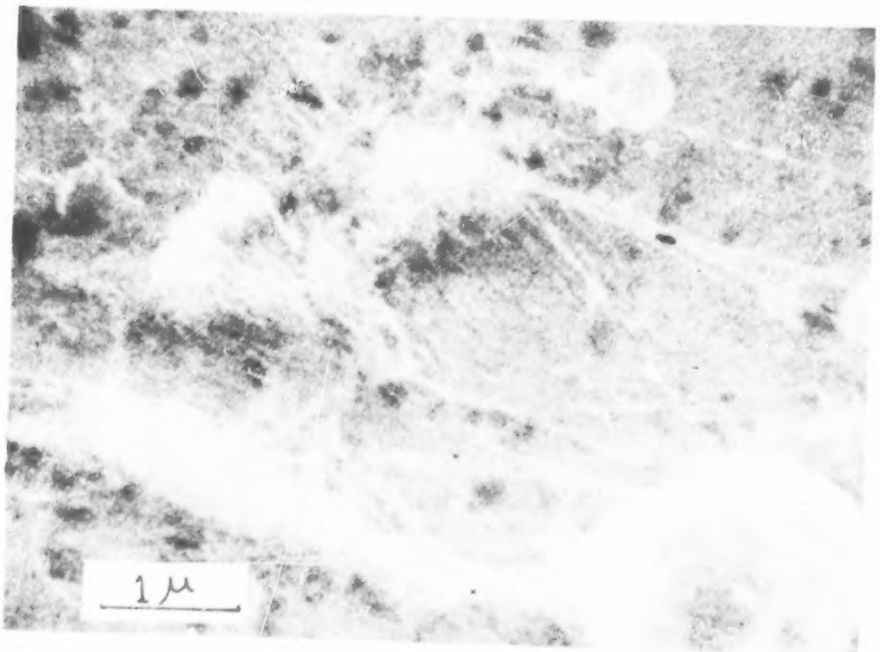

Figure 85. Scanning Electron Micrograph of a Human Chromosome. Note surface (arrow) where fiber-like contours have an orientation roughly parallel to each other. The apparent diameter of the fibers is about 1000 Å.

B. ANIMAL STUDIES

Effects of 2450 MHz Microwave Radiation on Cultivated Rat Kangaroo Cells

K. T. S. Yao, M. M. Jiles

The effects of acute 2450 MHz microwave exposure have been described with serially cultivated cells, which were originally derived from choroid and bone marrow tissues of the rat kangaroo (*Potorous tridactylis*). To prepare cell cultures for exposure, medium was drained from plastic T-30 flasks and the cell layer was rinsed with balanced saline. During exposure, the saline solution was drained from the cell layer. Microwave exposures lasted 5 to 30 min at distances of 10 to 50 cm in an anechoic chamber. At 50 cm a power density of 200 mW/cm^2 was measured with a 9 cm dipole antenna with a remote control. At the exposure times and distances used, balanced saline boiled when present.

The number of surviving cells was determined with hemacytometric methods at 24-hour intervals after irradiation. Figure 86 shows the microwave effects on cell growth during 6 days after irradiation. At 50 cm from the source, no effects on cell growth were observed. At 25 cm, cell growth was depressed after 20 and 30 min of exposure. At 10 cm, cell growth was depressed after each exposure used. Most of the cells in cultures irradiated at the 10 cm distance appeared dead.

Surviving cells in irradiated cultures from bone marrow were examined for chromosomal anomalies. Anomalies were observed only in cells irradiated for at least 10 minutes at the 10 cm distance. Both conventional types of aberrations and non-localized severe deterioration of chromosomes were observed. Table 31 shows the types and frequencies of aberrations. The frequency of aberrations increases with duration of exposure. On the other hand, the variation in the frequency of cells with non-localized chromosomal deterioration was not significantly different among the various exposures. Table 32 shows the frequencies of the abnormal cells with the non-localized chromosomal deterioration.

TABLE 31. MICROWAVE RADIATION INDUCED CHROMOSOME ABERRATIONS IN RAT KANGAROO BONE MARROW CELLS[a]

Minutes	Cells Observed	Chromatid Break	Isochromatid Break	Dicentric	Ring	Chromatid Exchange
0	400	0	0	0	0	0
5	150	0	0	0	0	0
12	50	0	1	1	0	0
15	333	3	15	3	1	0
18	150	3	5	1	0	0
20	111	1	8	1	0	0
Total	643	7	29	6	1	0

a. 10 cm from the open front of the oven.

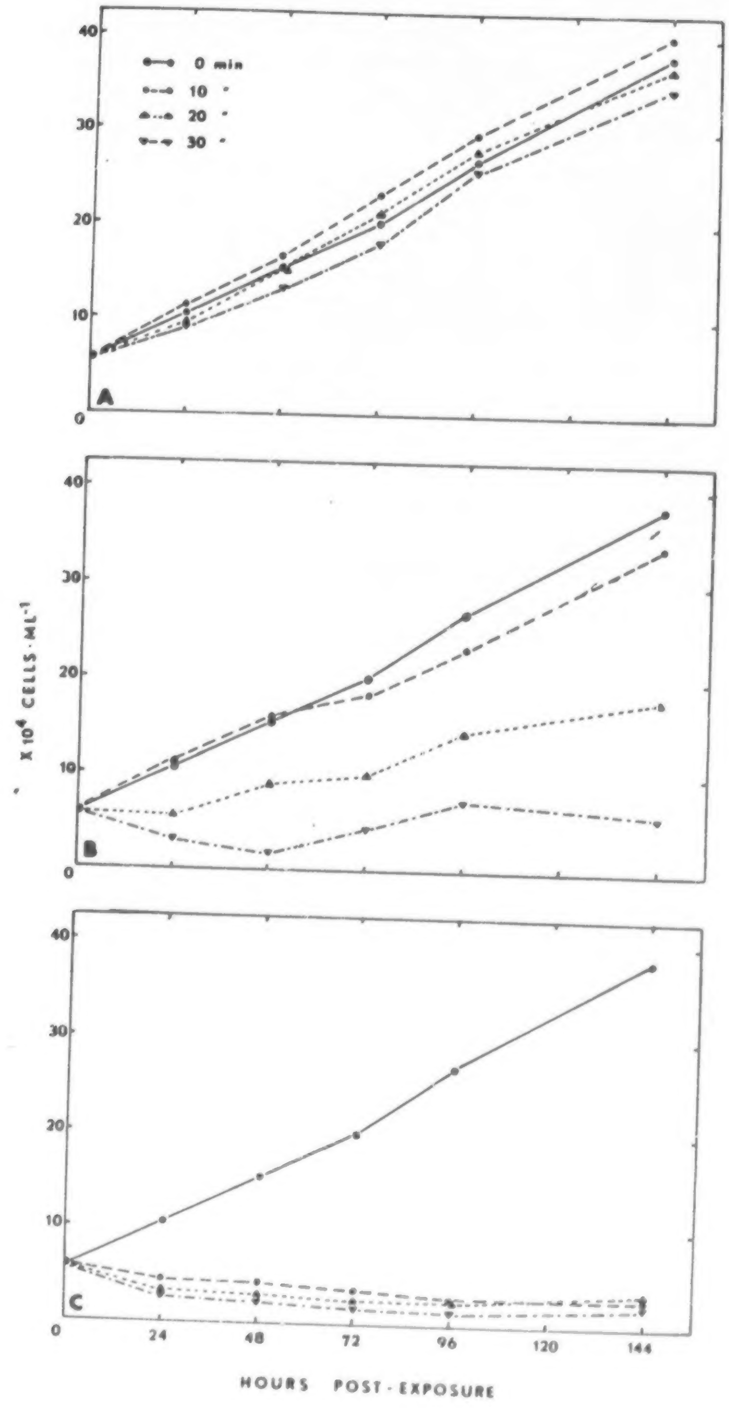

Figure 86. Cell Growth After 2450 MHz Microwave Irradiation of Cell Cultures from the Rat Kangaroo Choroid. Distance of the culture flask from the generator: (A) 50 cm; (B) 25 cm; (C) 10 cm. Duration of exposure as indicated.

TABLE 32. PERCENTAGE OF ABNORMAL MITOTIC CELLS IN RAT KANGAROO BONE MARROW CELLS[a]

Minutes of Irradiation	Hours after Irradiation			
	24	48	72	96
0	0	4	3	2
12	10	16	12	3
15	5	26	22	6
18	11	23	19	4
20	12	23	18	2

a. 200 Mitotic Cells Each Treatment.

Chromosomal aberrations were observed 8 hr after exposure. Peak frequency of aberrations occurred 48 hr after exposure, as shown in Table 33. Isochromatid breaks were observed most frequently and accounted for 29 of the 43 observed aberrations. The two longest chromosomes of the complement (chromosomes 1 and 2) contained 31 of the 43 observed aberrations. Incubation of cultures in 5-bromodeoxyuridine (BUDR) (25 μg/ml of medium) for 16 hr prior to irradiation modified the chromosomal response. Dicentrics were most frequently observed after BUDR treatment and accounted for 14 of 30 of observed aberrations. Furthermore, the No. 4 chromosome (a short metacentric) was the most frequently aberrant chromosome.

TABLE 33. NUMBER OF CHROMOSOME ABERRATIONS OBSERVED IN BONE MARROW CELLS AFTER MICROWAVE IRRADIATION

Hours	No. Cells Observed	Chromatid Break	Isochromatid Break	Dicentric	Ring	Chromatid Exchange
8	50	0	1	0	0	0
24	50	0	2	0	1	0
48	293	2	17	4	0	0
72	150	3	7	2	0	0
96	100	2	2	0	0	0
Total	643	7	29	6	1	0

Genetics of *Drosophila melanogaster* Exposed to 2450 MHz Microwave Radiation

E. C. Beyer, T. L. Pay

Because of the extensive genetic research on *Drosophila melanogaster*, the species was selected for examination of microwave effects on inheritance. This report summarizes the status of the study, which is intended to test the flies for genetic variations after exposures in both high and low power microwave fields.

Adult male wild-type flies were exposed less than 24 hours after ecdysis to 2450 MHz for 45 min. The power density was varied by varying the forward power of the generator. The flies were exposed in an anechoic chamber and the source-to-specimen distance was kept constant.

The immediate lethality of flies in the microwave field is shown in Figure 87 under two conditions of exposure. Flies in a gelatin capsule (40 flies/capsule) die at a slightly lower power density than do flies in a specially constructed acrylic plastic holder (10 flies/holder). The possible significance of the difference is not known.

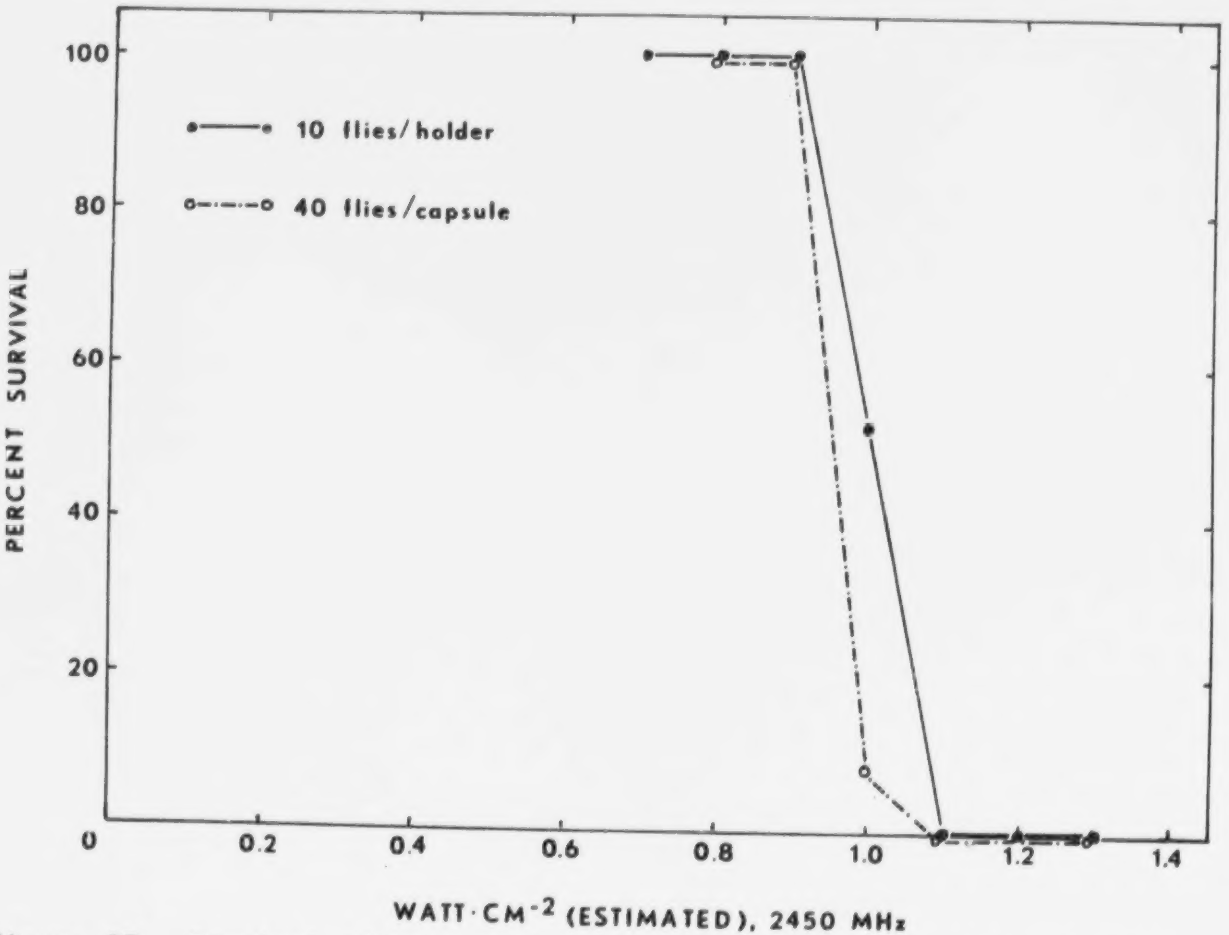

Figure 87. Survival of *Drosophila melanogaster* Males in 2450 MHz Microwave Fields. Duration of each exposure was 45 minutes. Power density was calculated from forward power at which generator was operated.

Surviving flies were mated with stock Muller-5 virgin females to determine genetic changes on the X-chromosome resulting from the irradiation. Table 34 summarizes results to date in the F_2 from the crosses. Two unexpected crossover offspring have been observed. Although the number of examined F_2 flies is small, the incidence of crossover offspring, 2/578 or 0.35 percent, appears to be high in comparison with the spontaneous incidence of crossover offspring in the M-5 strain.

It must be emphasized that the observed effects follow power densities that are close to lethal. It is therefore impossible to distinguish any specific microwave effects from the secondary thermal effects.

Chromosomal Analysis of Rat Mammary Tumor Cells in Culture

S. A. Knadle, L. J. Gillespie

Investigators in the Pathologic Studies Section have derived and maintained cells in culture from a papillary cystadenoma of the mammary gland of the Osborne-Mendel rat. The tumor developed six months after whole-body exposure of the rat to 450 R of X rays. Details of the cell cultures are summarized on p. 61. We report here progress in identification of chromosomal changes in the mammary tumor cells.

Cells from both early (second to seventh) and late (35th to 40th) serial passages were grown on cover glasses in Leighton tubes for chromosomal examination. Analysis is essentially complete for late passage cells. In RMT-A cells, which are epithelial, 76 percent show the diploid number of 42 chromosomes. In RMT-D cells, which are fibroblastic, 60 percent show the diploid number. The range of variation from the diploid chromosome number is greater in the RMT-D cells than in the RMT-A cells. Both lines show approximately 3 percent tetraploidy.

Fragmented chromosomes, mono- and nullosomies, and acentric fragments are common in RMT-D cells. Specific chromosomal changes appear to affect the No. 3 and the No. 13 chromosomes. On the No. 3 chromosome, anisochromia seems probable in 45 percent of cells in RMT-A and in 62 percent of RMT-D lines. The anisochromia, shown in Figure 88 may be present in analyzed cells in which the position of the No. 3 chromosome prevented careful measurements. An abnormal satellite of one of the No. 13 chromosomes was observed in 36 percent of RMT-A cells and in 86 percent of the RMT-D cells.

The significance of the chromosomal changes in determining the ability of the cell lines to induce tumors is unknown. It should be pointed out that the RMT-D cells show a greater degree of chromosomal change from the normal rat complement than do the RMT-A cells. Preliminary observations of induced tumors suggest that those induced by RMT-D cells persist longer in host rats than do tumors induced by RMT-A cells.

TABLE 34. DROSOPHILA MELANOGASTER IRRADIATED MALES USED IN GENETIC CROSSES WITH MULLER-5 STRAIN FEMALES

Power Density, W·cm^{-2}	Number of Irradiated Males	Number of Survivors for Mating	F_2 Offspring	F_2 Crossover Offspring, Sex, Crossover markers
0.7	10	10	150	1♀, Basc/asc
0.9	10	4	66	0
1.0	20	7	412	1♂, asc/Y

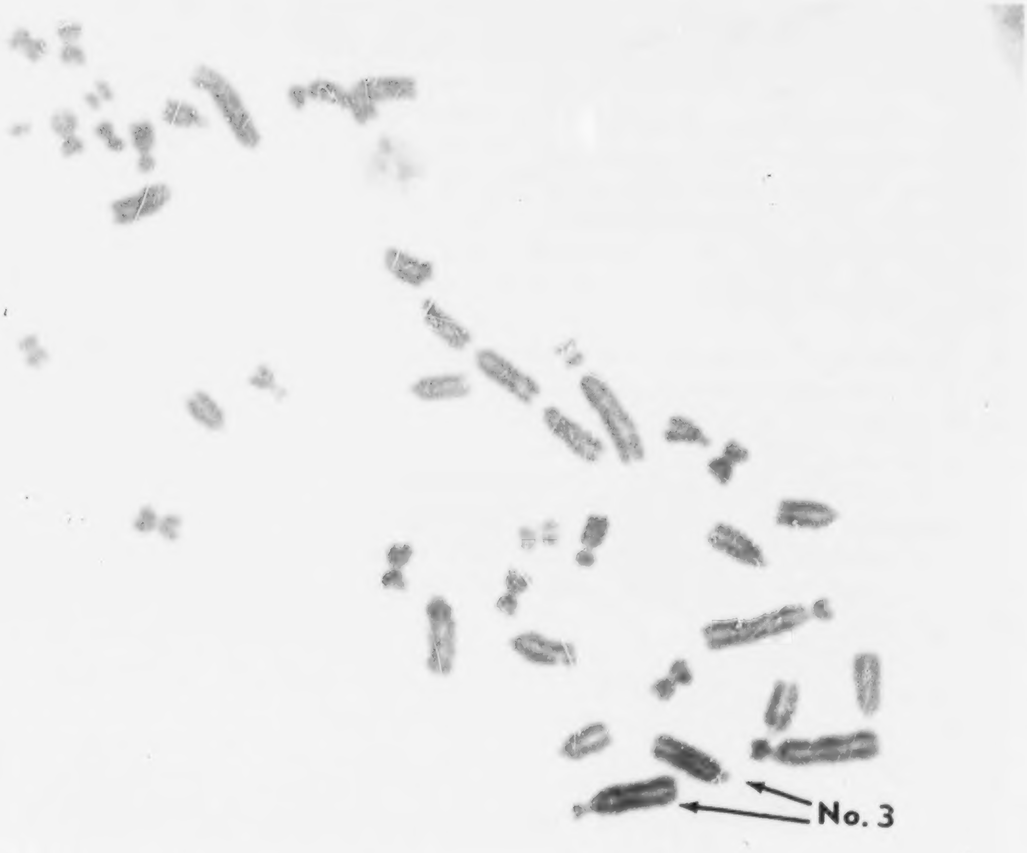

Figure 88. A Colcemid-Metaphase Chromosome Spread from RMT-A Cell Culture with Anisochromia of the No. 3 Chromosome (arrows). The cell is monosomic for the No. 10 chromosome.

Radiosensitivity of Haploid and Diploid Rat Kangaroo Cells Cultured from the Corneal Endothelium

K. T. S. Yao

In mammals the germ cells of the testis and ovary become haploid as part of maturation processes by which gametes become available for fertilization. Haploid cells from mammalian somatic tissues have been reported only in very early parthenogenetic embryos of the rat and mouse. In our cultivated cells which were originally derived from the corneal endothelium of the rat kangaroo, Potorous tridactylis, we have observed that more than 10 percent of the cells in mitosis have a haploid number of chromosomes. Figure 89 shows cells with diploid and haploid chromosome numbers. We have exposed cultures to X rays in order to compare the radiosensitivity of the haploid cells with the diploid cells.

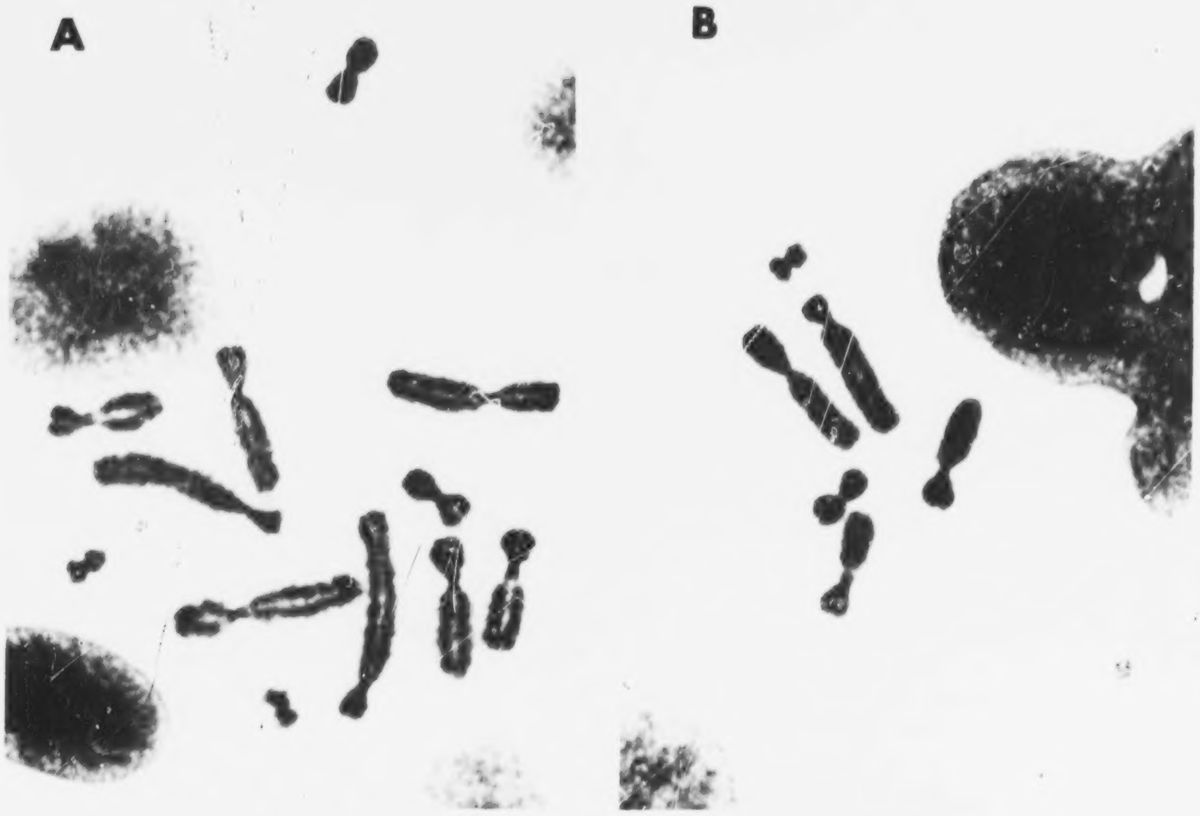

Figure 89. Colcemid-Metaphase Chromosome Spreads from the Rat Kangaroo Corneal Endothelium Cell Culture. (A) Cell with the normal diploid chromosome complement; (B) cell with a haploid chromosome complement.

The cell culture was initiated in February 1967, and has been serially subcultured for 42 passages to date. The cells are fibroblast-like and form multiple layers on culture surfaces. The cell population doubling time is about 16-17 hours. The cells grow in medium 199 supplemented with 20 percent fetal bovine serum, 10 percent NCTC-109 medium, 1 mM Na-pyruvate and 3 mM L-glutamine, and penicillin and streptomycin (100 units/ml of medium of each).

Cells were transplanted into plastic T-30 flasks 24 hours before X-ray exposure. Cells received 0 to 150 rads of X rays (250 kVcp, 15 mA, no added filtration, HVL equivalent to 0.55 mm Al, target-to-specimen distance, 119.7 cm, dose rate 31.3 rads per minute). Cell cultures were fixed 6, 12, 24, 36, and 48 hours after irradiation. Colcemid was added to each culture 3 hours before fixation. Cells were stained with Giemsa, and 50 haploid and 50 diploid cells in each preparation were examined for chromosome aberrations.

Table 35 shows the relative frequency of cells with a haploid chromosome number. In general, the frequency of the haploid cells tends to increase with both age of culture and X-ray dose, with the exception that the haploid frequency is as low at 36 hours as at 6 hours post-irradiation. It is possible, therefore, that the apparent relationship between frequency of haploid cells and X-ray dose may simply reflect cell cycle effects of the irradiation. Tables 36 and 37 show the incidence of aberrations in haploid and diploid cells, respectively. The number of aberrations shown is the sum of aberrations seen in the sampling intervals after each X-ray dose. Exchange-type aberrations were not observed. The data indicate that aberrations were more than three times more frequent in the diploid than in the haploid cells.

TABLE 35. RELATIVE FREQUENCY OF CULTURED CELLS WITH A HAPLOID NUMBER OF CHROMOSOMES

Hours after Irradiation	Number of Haploid Cells per 50 Diploid Cells at Each X-ray Exposure					
	0 rads	20 rads	50 rads	100 rads	150 rads	Average
6	24	11	20	--	--	18.3
12	--	21	24	16	--	20.5
24	16	26	20	30	35	25.4
36	--	13	17	19	--	16.3
48	22	32	44	47	52	39.4
Average	20.7	20.6	25.0	28.0	43.5	

TABLE 36. INCIDENCE OF X-RAY-INDUCED CHROMOSOMAL ABERRATIONS IN CELLS WITH A HAPLOID CHROMOSOME NUMBER

X-Ray Dose, Rads	Cells Scored	Chromosomal Aberrations				Calculated Number of Breaks	Breaks per Cell	Breaks per Cell per Rad
		C'tid Breaks	Iso'tid Breaks	Dicentrics[a]	Rings[a]			
0	150	0	0	0	0	0	0	0
20	250	1	0	9	2	23	0.092	0.0046
50	250	1	0	4	2	13	0.044	0.0009
100	200	2	3	5	2	19	0.095	0.00095
150	100	0	2	5	1	14	0.140	0.00093
X-Ray Total		4	5	23	7	69	0.086	

a. Counted as requiring two breaks.

TABLE 37. INCIDENCE OF X-RAY-INDUCED CHROMOSOMAL ABERRATIONS IN DIPLOID CELLS

X-Ray Dose, Rads	Cells Scored	Chromosomal Aberrations				Calculated Number of Breaks	Breaks per Cell	Breaks per Cell per Rad
		C'tid Breaks	Iso'tid Breaks	Dicentrics[a]	Rings[a]			
0	150	0	0	1	0	2	0.013	--
20	250	3	2	30	3	71	0.284	0.0142
50	250	1	5	18	8	58	0.232	0.0046
100	200	9	9	25	1	70	0.350	0.0035
150	100	4	5	14	0	37	0.370	0.0025
X-ray Total		17	21	87	12	236	0.295	

a. Counted as requiring two breaks.

Observations on Cell Cultures from Adult Chinese Hamsters

L. J. Gillespie, W. M. Leach

Cell cultures were initiated with the thyroid glands from an 18-month-old male Chinese hamster (Cricetulus griseus). Between the second and fourth in vitro serial passage of the cells a marker chromosome appeared. As shown in Figure 90, karyotypic analysis of the cells places the marker in the group of subtelocentric chromosomes of the hamster complement, probably as the No. 5 chromosome.

To test the stability of the marker, the fourth passage cells were hybridized with thyroid cells from both 7-day-old and 1-year-old Chinese hamsters. The hybrids between cells from adult animals resulted in a substantial proportion of mixed polyploid cells in the population. The cells were hexaploid in the No. 1 - 4 chromosome group, in the No. 8 - 10 group, and perhaps in the sex chromosomes. The No. 5 - 7 group was octaploid and contained one marker per diploid set. Figure 91 shows the karyotype of the adult : adult hybrid. The mixture of cells from the adult and young animals resulted in tetraploid cells in the resultant cell population. The marker was not duplicated in the second diploid set. However, a second marker was obtained, one of the four No. 1 chromosomes. The karyotype of the adult : young hybrid is shown in Figure 92.

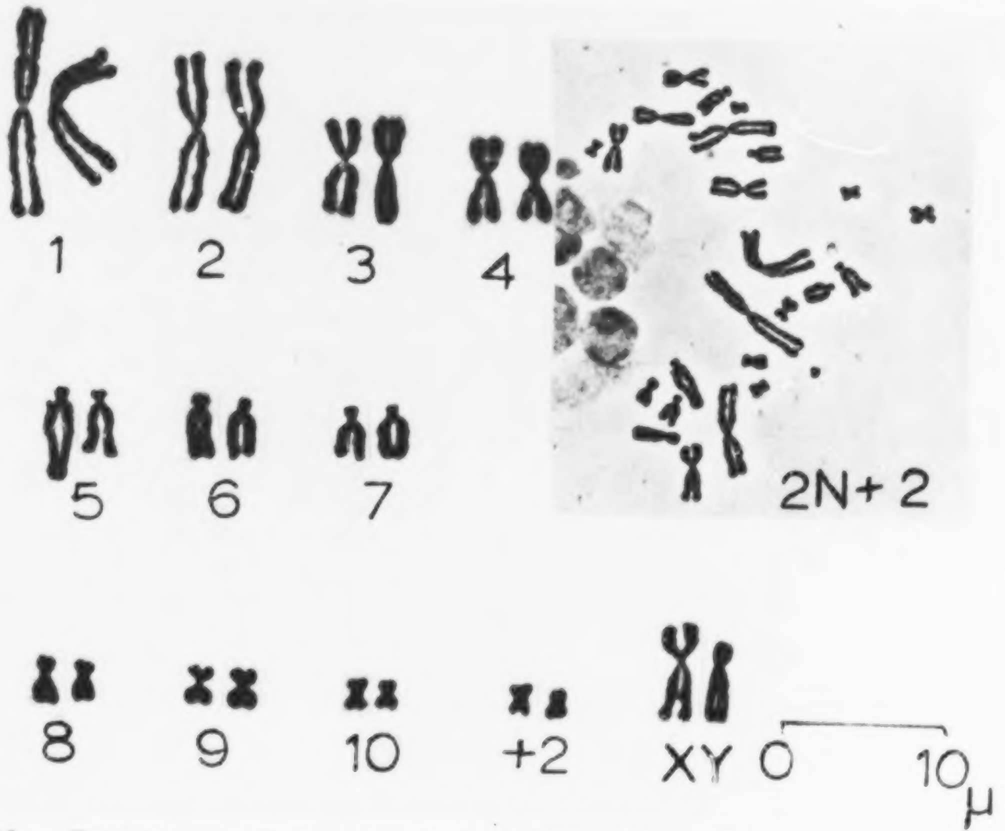

Figure 90. Karyotype of Passage Cells from Adult Chinese Hamster Thyroid Showing Marker Chromosome.

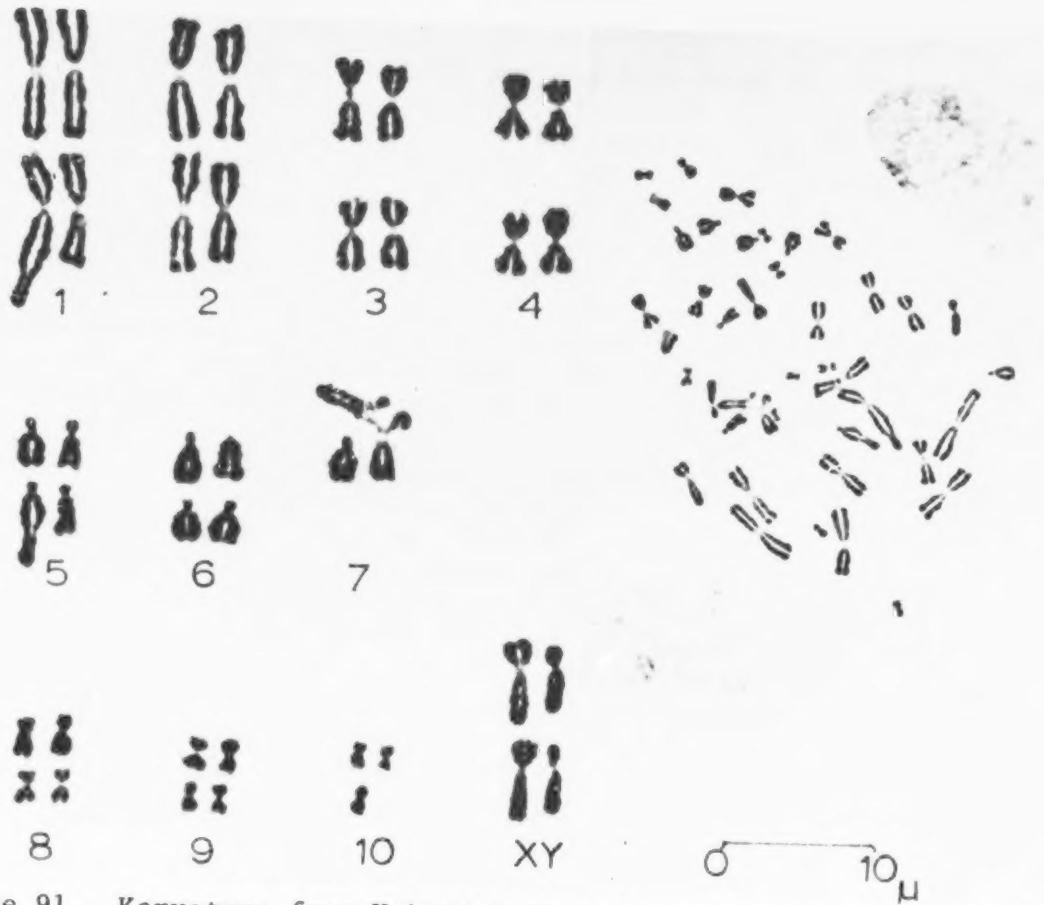
1
2
3
4
5
6
7
8
9
10
XY
0
10μ

Figure 91. Karyotype from Hybrid Cell Cultures (Adult : Adult) from Chinese Hamster Thyroids.

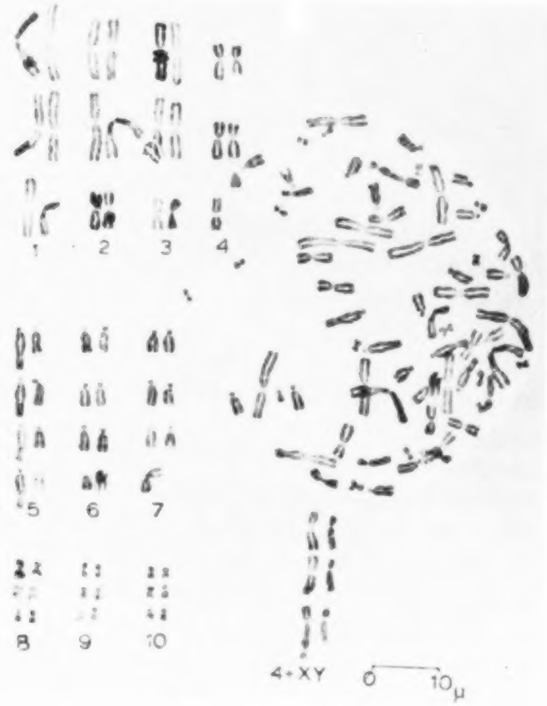
1
2
3
4
5
6
7
8
9
10
4+XY
0
10μ

Figure 92. Karyotype from Hybrid Cell Cultures (Adult : Young) from Chinese Hamster Thyroids.

Cells from the 18-month-old hamster were reaggregated at the 30th in vitro serial passage. Growth characteristics were observed in cultures prepared from the reaggregated cells. Outgrowths of discrete colonies of cells were segretated into basically two types of cells as shown in Figure 93A. At 3-5 days of culture, isolated giant cells appeared in the colony outgrowths (Figure 93). The giant cells were highly granular, sometimes with a vesiculated cytoplasm. Occasionally giant cells were connected with surrounding, apparently normal, cells by cytoplasmic strands. Figure 94 shows examples of giant cells in fixed and stained preparations. The nucleus appears segmented in some giant cells. Examples of giant cells in unfixed preparations are shown in Figure 95.

An Unusual Chromosome Constitution in a Family of Cats

L. J. Gillespie

During the development of a simplified technique for culturing cat peripheral leukocytes, it was observed that the test animal had a chromosome complement with a secondary constriction or heterochromatic region in the median to subterminal portion of the long arm of a chromosome from the No. 3-4 group. Examination of the cat's sire and one surviving sibling revealed that the anomaly was present in them as well. Figure 96 shows the cat chromosomes with the anomalous region. A less frequent isochromatid gap or heterochromatic region was also present in the No. 1-2 group of chromosomes from the three animals.

Modifications of X-Ray-Induced Cell Death by Drugs

M. M. Jiles, K. T. S. Yao

The concomitant use of drugs and X radiation has substantial apparent value in certain human therapeutic procedures. Moreover, the growth of drug use (and abuse) in the United States raises questions concerning the possible interactions of drugs with ionizing radiation that may result in magnification of the mutagenicity of the radiation. A convenient and rapid method to screen drugs for possible interaction with radiation is therefore needed. This report summarizes data obtained in tests of a possible method. The attractiveness of the method is its relative convenience, in that the total test time is two days. The assay is based on staining of cultured cells.

Cells, serially cultured from the corneal endothelium of the rat kangaroo (Potorous tridactylis), were grown on cover glasses in plastic T-30 flasks in a medium of 4 parts Waymouth's medium, 4 parts medium 199, and 1 part medium 109, supplemented with 20 percent fetal bovine serum, 1 percent Na-pyruvate and 1 mM L-glutamine, plus 100 units of penicillin and 100 μg streptomycin per ml of medium. The cells were transplanted to the flasks 24 hours before X-ray exposure either to 0 or to 300 rads. Either 16 hours before, or immediately after irradiation, the cells were treated with one of the following drug concentrations: Actinomycin-D, 4 μg/ml; 5-bromodeoxyuridine, 56 μg/ml; Hadacidin, 440 μg/ml, and Colcemid, 30 μg/ml. The cultures were fixed 24 hours after irradiation, and stained with May-Grunwald and Giemsa. The frequency of "dead cells" was determined. A cell with a darkly stained nucleus and/or cytoplasm was scored as being a dead cell.

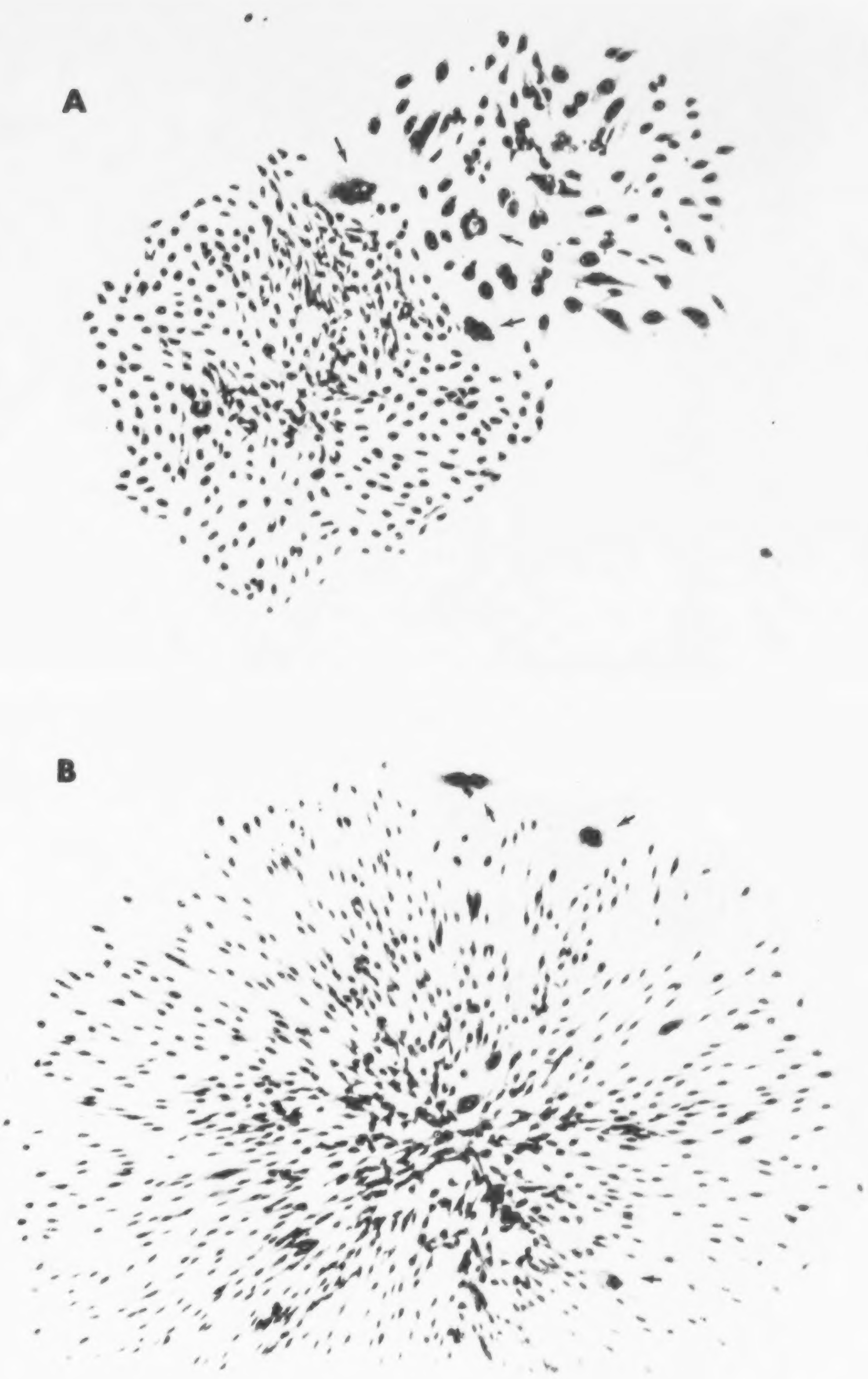

Figure 93. Colonial Growth of Thyroid Cells after Reaggregation at the 30th Serial Passage. Giant cells (arrows) form 3 to 5 days after reaggregation. (A) Two discrete cell types are segregated.

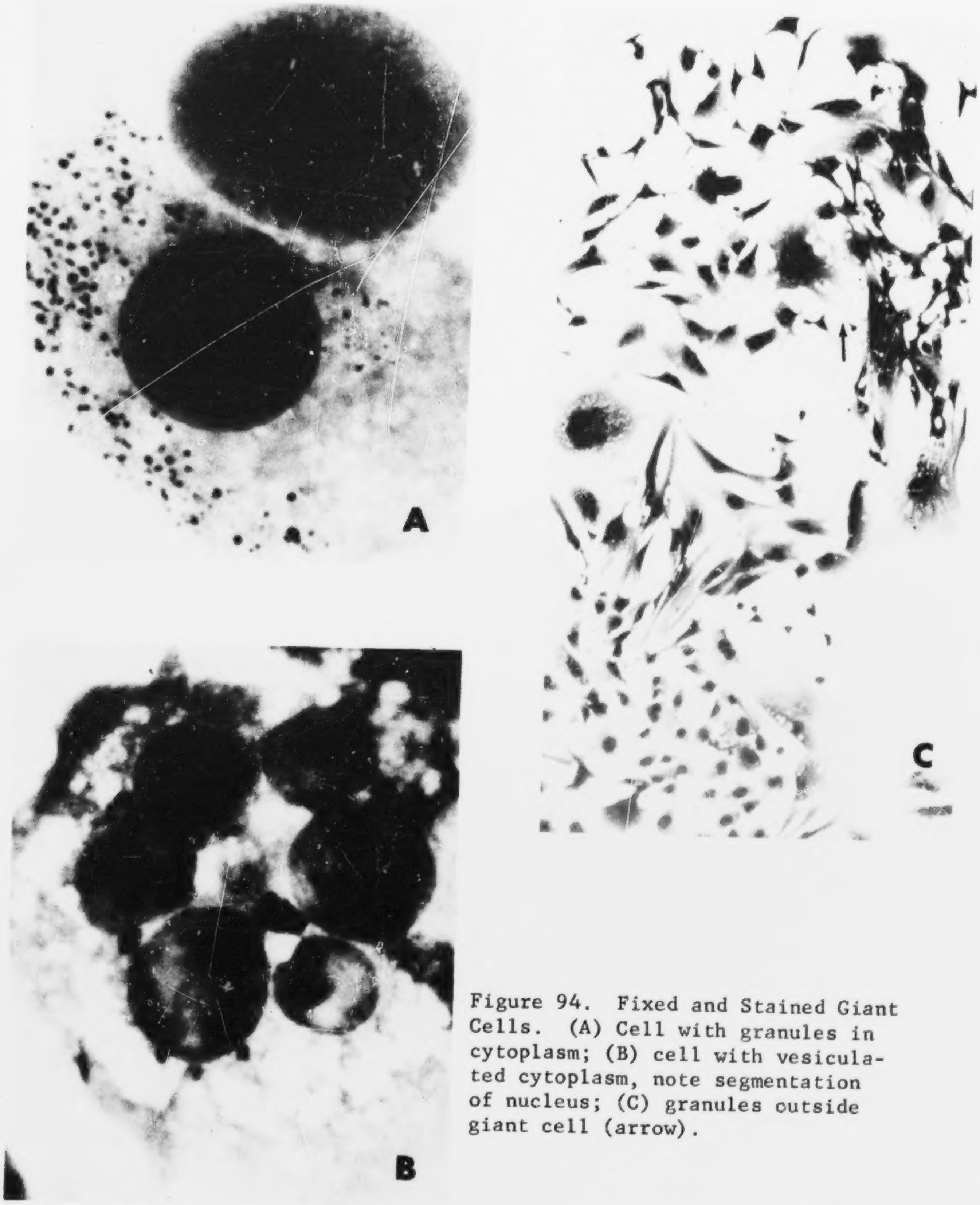

Figure 94. Fixed and Stained Giant Cells. (A) Cell with granules in cytoplasm; (B) cell with vesiculated cytoplasm, note segmentation of nucleus; (C) granules outside giant cell (arrow).

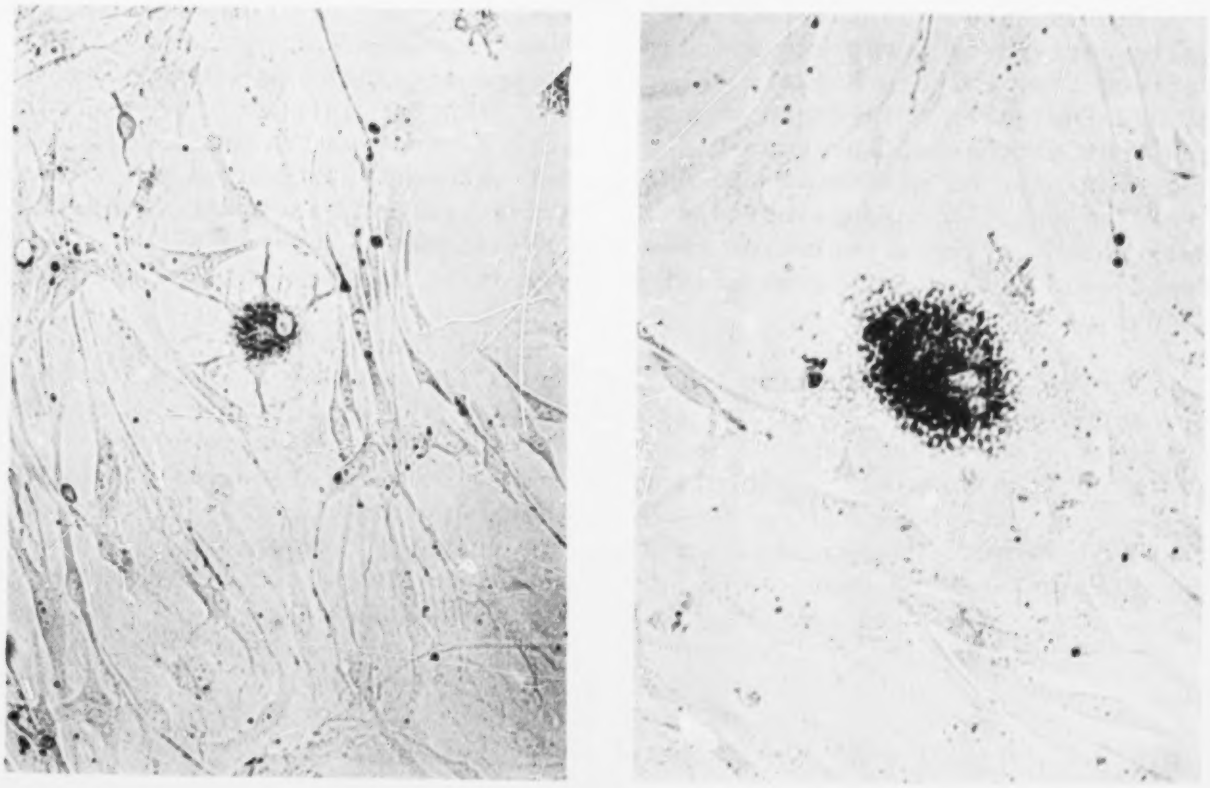

Figure 95. Unfixed Cultures with Giant Cells.

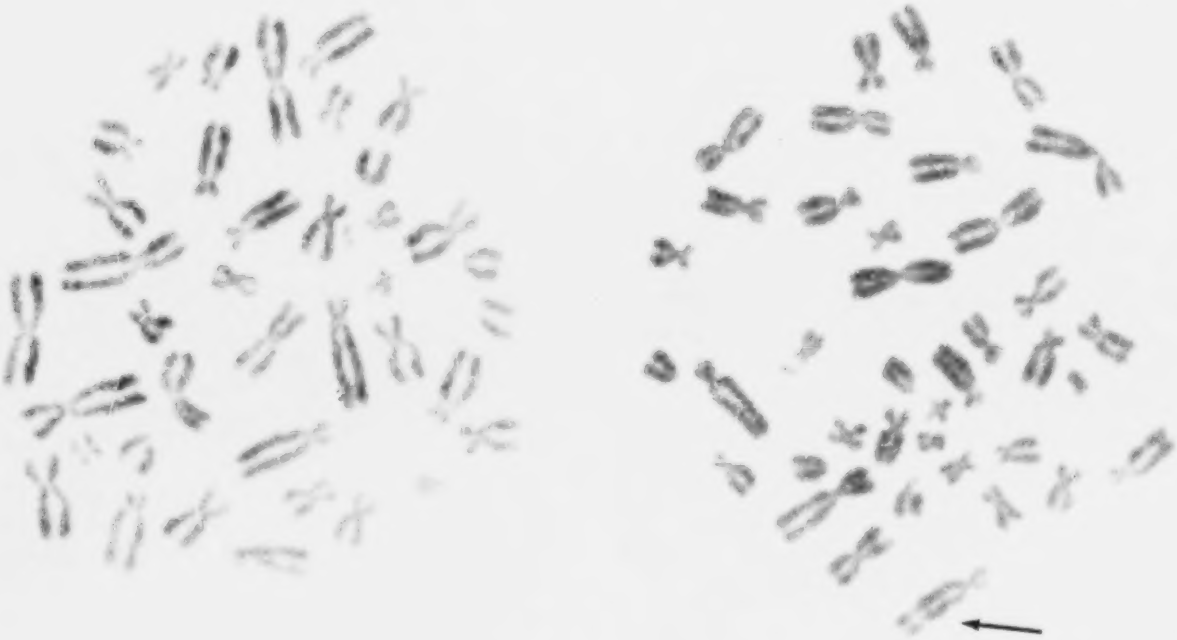

Figure 96. Chromosomes from Cat Peripheral Leukocyte Cultures. (A) Usual chromosomal constitution; (B) unusual familial chromosomal constitution. Arrow indicates unusual chromosome.

Tables 38 and 39 tabulate the results of seven experiments, six with cells derived from rat kangaroo corneal endothelium and one with cells derived from Chinese hamster spleen. It appears that the irradiation exposure increases cell death in comparison with the unirradiated control, and that neither actinomycin-D nor Hadacidin causes additional cell killing when used as pre-treatment and post-treatment. When used prior to irradiation, 5-bromodeoxyuridine may have a protective effect against cell death; after irradiation the chemical seems to have no effect. Colcemid used either before or after X irradiation appears to cause additional cell killing.

The single most striking observation on the data is the wide variability of response between the replicates. A variety of speculative hypotheses might be advanced to account for the observed variability. Three obvious possible explanations are drug concentration, differential release of dead cells from the cover glass, and differential effects on the cell cycle. On the basis of the data on hand, it appears that the experimental method under test is not sufficiently reproducible to permit routine screening for drug-radiation interactions.

A Microtechnique for Chinese Hamster Blood Cultures

L. J. Gillespie, W. M. Leach, B. C. Ward

A technique for culturing Chinese hamster (Cricetulus griseus) blood has been developed to permit repetitive sampling of individual animals. The technique uses unseparated whole blood (0.05-0.4 ml, depending on animal age) obtained from the orbital venous sinus with heparinized capillary pipets. From the pipet, 1-2 drops of blood are added to 5 ml of the culture medium, Trowell T8 supplemented with 30 percent fetal bovine serum and 100 units of penicillin and 0.01 ml of poke weed mitogen per ml of medium. Culture tubes are incubated without disturbance at 37° C for 56-60 hours or 38° C for 48-50 hours. Mitotic cells are collected with 0.2 µg/ml Colcemid near the end of the culture period.

The success of cultures appears to depend on the medium used, the amount of blood used, and temperature of incubation. Approximately 3-5 mitoses are found per 100 small lymphocytes in 48-hour-cultures at 38° C. Although the same cultures show about 40 percent of cells in blast transformation (determined by cell size), degenerating cells are evident in cultures held for 56-60 hours at 38° C.

TABLE 38. "CELL DEATH" IN RAT KANGAROO CORNEAL ENDOTHELIUM DERIVED CULTURES AFTER CHEMICAL AND X-RAY TREATMENT[a]

Drug	Percent of Dead Cells after Irradiation								
	Replicates with 0 Rads				Replicates with 300 Rads				
	1	2	3	4	1	2	3	4	Aver.
None	25.8	9.1	7.2	15.6	18.3	43.6	7.6	25.8	23.8
Actinomycin-D	-	-	-	4.1	29.2	41.5	16.0	8.4	23.8
5-Bromodeoxy-uridine	-	-	-	11.3	12.3	12.3	-	5.4	10.0
Colcemid	-	-	-	62.2	66.7	42.3	34.1	14.1	39.3
Hadacidin	-	-	-	9.6	10.1	59.2	13.5	14.7	26.4

a. Cultures treated with drugs for 16 hours before X irradiation.

TABLE 39. "CELL DEATH" IN CULTURES EXPOSED TO DRUGS AFTER X-RAY EXPOSURE[a]

Drug	Percent of Dead Cells after Irradiation						
	Replicates with 0 Rads			Replicates with 300 Rads			
	1[b]	2	3	1	2	3	Aver.
None	16.7	20.4	-	24.5	19.9	38.0	27.5
Actinomycin-D	-	-	18.2	20.8	44.7	15.9	27.1
5-Bromodeoxy-uridine	-	-	16.2	18.9	26.4	24.5	23.3
Colcemid	-	-	88.1	67.3	29.6	78.8	58.6
Hadacidin	-	-	33.3	17.2	20.0	32.4	26.5

a. Cultures treated with drugs for 24 hours after X irradiation.

b. Cultures of cells derived from Chinese hamster spleen.

In Vitro Meiosis of Chinese and Golden Hamster Oocytes

K. T. S. Yao, E. W. Chu[1]

In female mammals meiotic activity may begin during fetal development, but becomes arrested until the immature oocyte is ovulated. The X-ray response of mammalian oocytes is, therefore, likely to be determined confidently in vitro. The purpose of this study was to stimulate the completion of meiosis in mammalian oocytes in vitro, so that the oocyte chromosome can be visualized microscopically. The study was conducted collaboratively with the National Cancer Institute, NIH.

Female Chinese and golden hamsters (Cricetulus griseus and Mesocricetus auratus, respectively) were sacrificed by cervical dislocation. The ovaries were dissected out asceptically and rinsed with Hanks balanced salt solution. To release oocytes from follicles, the ovaries were gently torn into small pieces with two needles. Oocytes were transferred with micropipettes to culture medium 199, supplemented with 20 percent fetal bovine serum plus 100 units penicillin and 100 μg streptomycin per ml of medium. Some oocytes were removed at 3-hour intervals for aceto-orcein squash preparations.

Meiotic activity is resumed in a substantial proportion of oocytes released in vitro. In most samples, oocytes can be identified at various stages of the first meiotic division. Within limits, later samples appear to contain a greater variety of meiotic stages than the earlier samples. Figure 97 shows a typical oocyte in culture. Examples of stages of meiosis from oocytes in culture are shown in Figures 98 and 99. Figure 99 compares metaphase I from the oocyte with that from a spermatocyte in a direct preparation of the Chinese hamster testis. In the spermatocyte, the No. 3 chromosome pair is in end-to-end pairing. In the oocyte, the No. 3 pair is in side-by-side pairing.

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[1] Address: Pathologic Anatomy Branch, General Laboratories and Clinics, National Cancer Institute, Bethesda, Maryland.

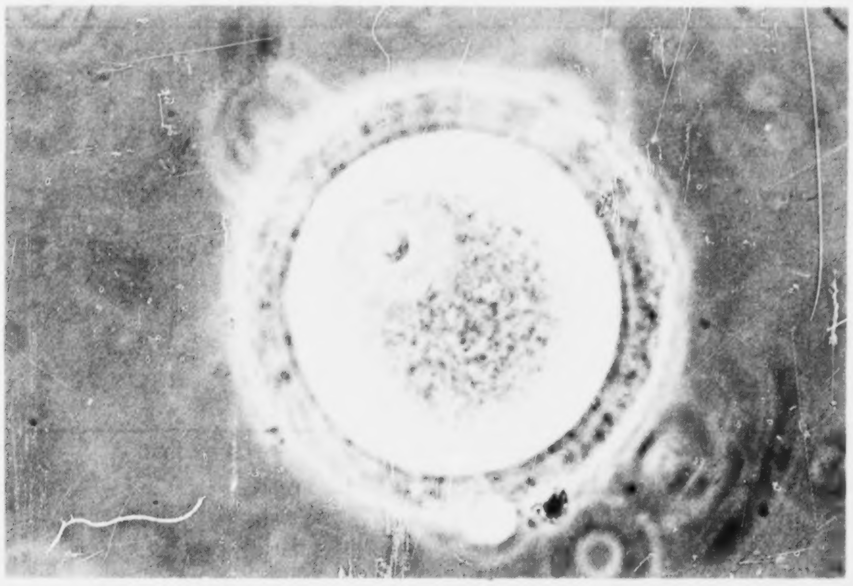

Figure 97. Photomicrograph of a Chinese Hamster Oocyte in Culture.

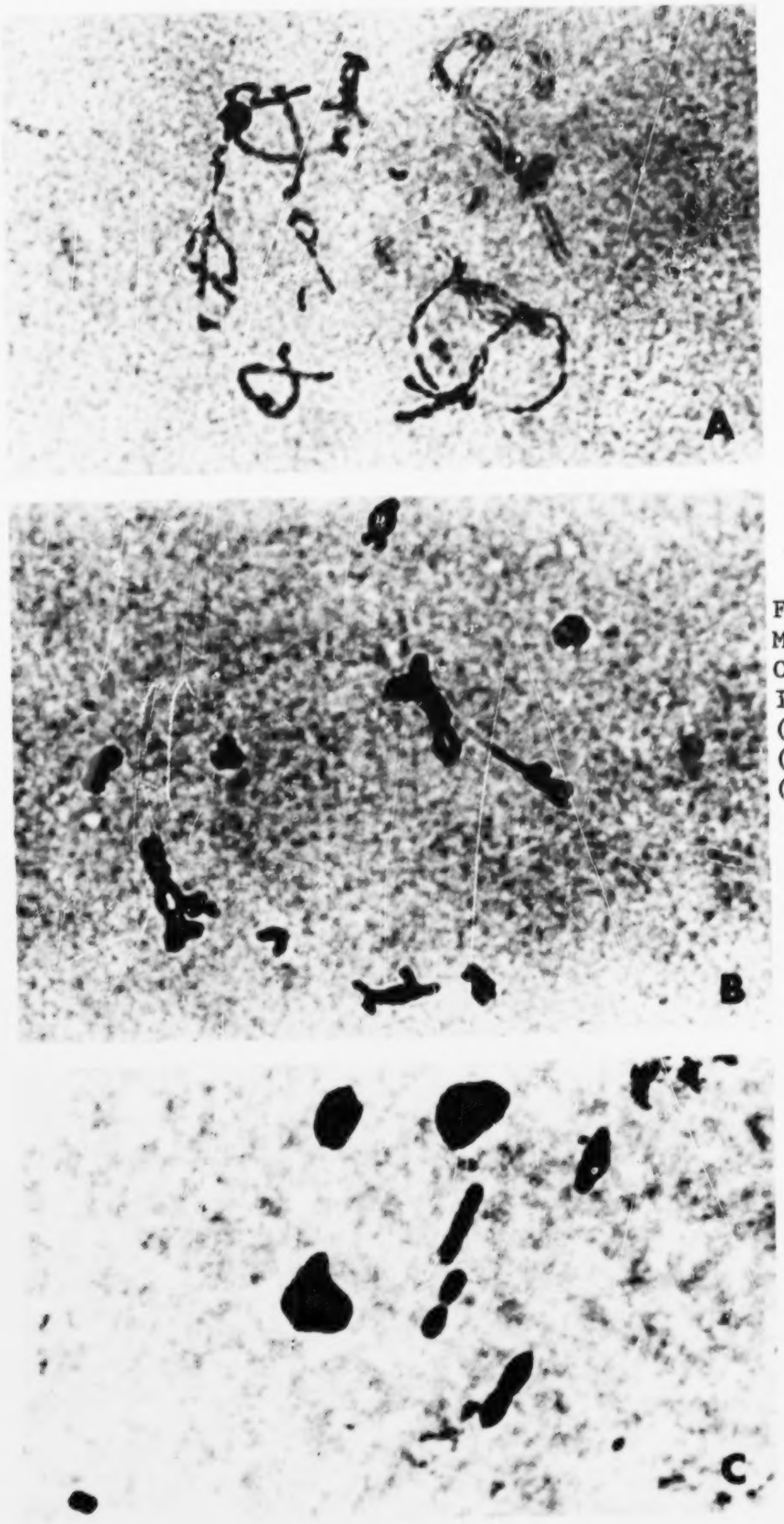

Figure 98. Stages of Meiosis Induced in Chinese Hamster Oocytes In Vitro.
(A) Pachynema
(B) Diakinesis
(C) Anaphase I

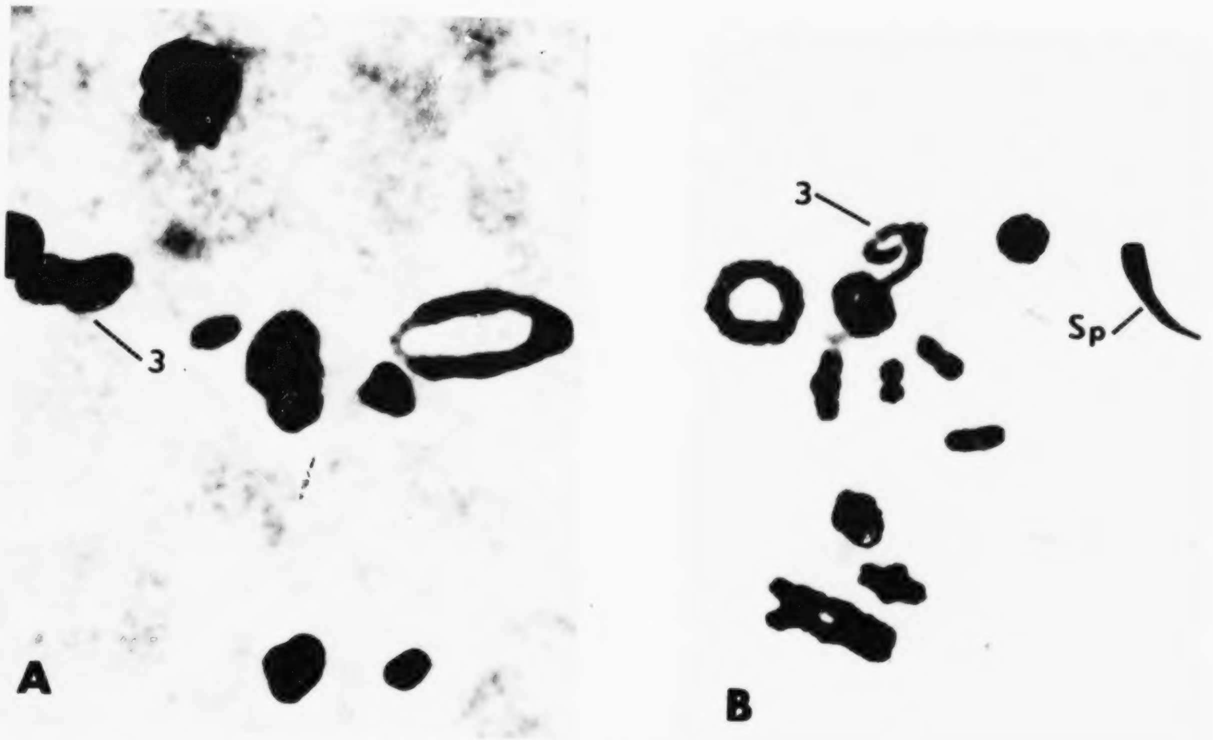

Figure 99. Metaphase I of Meiosis in Chinese Hamsters. The No. 3 chromosome determined on the basis of relative size. (A) In vitro oocyte, No. 3 chromosome in side-by-side pairing. (B) Spermatocyte from direct preparation, No. 3 chromosome in end-to-end pairing. Sp, spermatazoan head.

COLLABORATIVE RADIOLOGICAL HEALTH LABORATORY[1]

Dr. R. John Garner, Director

A. INTRODUCTION

The Collaborative Radiological Health Laboratory was conceived in 1962 as a joint operation between the sponsoring agency, the Division of Biological Effects of the Bureau of Radiological Health, U.S. Public Health Service, and the Department of Radiology and Radiation Biology, Colorado State University. Its primary purpose is to conduct a long-term study of the effects in the beagle of a single exposure to low levels of gamma radiation with emphasis on the investigation of the radiosensitivity of the unborn and young animal in terms of long-term effects.

The long-term experiment which constitutes the core program of the laboratory was initiated in 1967. Commitment of animals is ahead of schedule; over one quarter of the projected total of 1,700 dogs having been entered into the experiment by November 1969. It is nevertheless anticipated that it will be another 3 to 4 years before all animals are assigned. Protocols have been developed for longitudinal study of a number of parameters in all or sample populations of these animals. Examples are schedules for collecting data on body weight, head dimensions, and the time course of dental eruption (in connection with growth studies) and for assessing fertility in all males and breeding capacity in all females assigned to the experiment. Eye lesions are anticipated in some animals as they age. The data collected to date are insufficient to be of significance; however, together with the results obtained from an ancillary experiment in which dogs received heavy doses of gamma radiation to one half of the head, they tend to confirm that eye lesions are likely to occur only in animals exposed prenatally.

In an attempt to obtain a preview of effects in general that might be expected after many years in the long-term dogs, a pilot age-sensitivity study was initiated during 1968. As projected, this study will involve the examination for up to 4 years of about 300 animals which have survived a prenatal or neonatal exposure in the LD_{50} range. By November 1969, 117 animals had been entered into this experiment, and 32 scheduled for sacrifice at 70 days had been necropsied. These animals are also intended to provide further material for many of the feasibility and short-term effects studies described in Section C.

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[1] A detailed account of the work at the Collaborative Radiological Health Laboratory is given in the Annual Report for 1969, Colorado State University - U.S. Public Health Service Collaborative Radiological Health Laboratory, Fort Collins, Colorado, (Contract PH 86-62-135).

Whenever possible, to economize on the number of dogs needing to be maintained, experiments are coordinated; animals assigned to the pilot age-sensitivity study are, in fact, survivors from a more broadly-based lethality study designed, on the one hand, to give an indication of the radiosensitivity of the developing beagle in terms of acute lethality and, on the other, to provide base data for a recovery experiment. Emerging from this work is evidence that variations in radiosensitivity with age in the young beagle resemble those described in the mouse rather than the rat; the relative resistance of the neonatal beagle ($LD_{50/60}$ 406 R at 2 days, compared with 316 R at 1 year) is quite remarkable. In these studies, as in the long-term experiment, a constant exposure time rather than a constant exposure rate has been adopted. A small exposure rate study has shown that, at least in terms of the $LD_{50/60}$, variations in exposure rate of 4 - 32 R per min. are unlikely to be of practical significance.

As part of a sustained effort to devise techniques applicable to longitudinal studies in the long-term animals (in which only relatively non-stressful methods of testing are permissible) the possibility of determining auditory acuity in the beagle has been investigated. Loss in ability to perceive the higher frequencies is a well-known age-related phenomenon in man. Two techniques have been applied, the recording of acoustically evoked potentials from the scalp (which requires application of sophisticated analytical methods) and a more conventional behavioral avoidance acoustical conditioning approach. Preliminary results provide good auguries for the former and emphasize the time-consuming nature of the latter.

An organ to which it is proposed to devote considerable effort in the long-term studies is the kidney. Preliminary work needed to refine methods for studying morphological changes has been completed and fine-structure studies are now underway. The few problems remaining before adoption as a routine procedure of a method for estimating parameters of renal function involving a single injection of ^{125}I-hippuran and subsequent external counting (designed to replace prohibited conventional constant infusion techniques) have been resolved. So also has the problem of obtaining a urine sample "on demand" from a dog; a simple electrical stimulation technique has now been developed for this purpose.

Concurrently with the long-term experiment, an attempt is being made to define the acute injury following irradiation during early development. The prenatal response of the beagle to a single exposure of 150 R gamma radiation between the 8th and 35th day of gestation (preimplantation to early fetal period) has been characterized. In terms of prenatal mortality, sensitivity was greatest shortly after implantation but still appreciable towards the end of the embryonic period. The most sensitive time for production of malformations occurred slightly later than that for peak mortality.

Progress in a study to assess recovery in the embryo, fetus, and neonate can be expected to be slow since it is designed to absorb animals produced for but not assigned to the long-term experiment for various reasons; it has, however, been satisfactory.

As a result of the need to utilize non-invasive methods of assessing CNS function, mathematical methods of analysis applicable to recordings of electrical potentials from the scalp have been developed. Fourier integral techniques applied to analysis of electroencephalograms have revealed changes in brain function a year after exposure of beagles as late fetuses to high doses of gamma radiation.

Studies on the effect of prenatal exposures in the LD_{50} range on various aspects of renal function have indicated a possible depression in glomerular filtration rate and tubular maximum for p-aminohippurate after about the 3rd month of postnatal life. Irradiated animals showed a depressed concentrating capacity but only during the first 2 weeks after birth. The possibility is being pursued of using enzyme ontogenesis as a model for studying moderately delayed effects of radiation and for identifying their loci.

The response of mechanisms responsible for the increase in glycerol phosphate dehydrogenase activity in liver (as indicated by enzyme activity in the 55 day-old beagle fetus) was found to differ in animals irradiated on the 18th and 21st day of gestation. The liver is absent as such in the 18 day-old embryo but present by 21 days; there is thus a strong suggestion that a direct effect upon the liver is involved. Since dogs are uneconomical animals to use in such studies, a system has been developed for assessing the early effects of irradiation upon the rate of appearance of hepatic serine dehydrase in prematurely delivered rats.

Although no scientific contributions have yet been made, this laboratory has entered into the area of non-ionizing radiations. Facilities are now available for studies on microwave effects on the CNS of dogs.

From the numerous ancillary studies necessitated by the long-term experiment, data have accumulated on various aspects of normal development in the beagle. Perhaps the contribution of greatest interest is a description of the prenatal developmental stages, derived from a study of 383 embryos and fetuses. Feasibility studies have yielded information on growth of the lumbar spine, development of frequency components in the electroencephalogram, development of the visual evoked potential and changes with age in pulse-wave velocity in the descending aorta. Light and electron microscopic techniques have been applied to characterize development of the kidney. The capacity of the renal tubules to reabsorb inorganic phosphate has been shown to be heavily taxed during the first 6 months of life.

The major cost of operation of this laboratory is maintenance of the cesarian-derived, controlled-access colony and supporting programs in pathology, clinical pathology, and biometry. Despite uncompromising culling of all animals for which there is no immediate requirement, the colony increased in size to a maximum of approximately 1,900 animals. Of these some 1,350 were assigned to experimental studies, the remainder representing animals maintained for breeding or weanling pups.

Thyroiditis, cardiopulmonary disease and glomerulonephritis remained the important conditions occurring spontaneously in the colony. Polyarthritis appeared for the first time; what is suspected to be pregnancy toxemia was encountered; and an investigation into the possibility of association of ^{125}I-hippuran with renal inflammation, while clearing the pharmaceutical, revealed a disconcerting increase in the incidence of this condition over the past 3 years. In addition, hepatic abscessation has been characterized in young puppies.

The ancestral composition of the colony has been analyzed in terms of a defined base colony. Data listings found to be necessary for day-to-day management have been inventoried. Attention is drawn to a defect in a TLD reader which could have led to possible errors of ± 10 percent in estimation of exposures in the order of those used in the long-term experiment.

During 1968, a new Research Section, Electrophysiology, was created. The purpose was twofold: to provide a nucleus of personnel for initiating microwave studies and to develop methods for quantifying physiological parameters measured by non-stressful procedures in studies with both ionizing and non-ionizing radiations. Historically, the tendency has been to interpret physiological data qualitatively; however, since many electrophysiological and neurophysiological data can be characterized as random processes, they should be amenable to application of the techniques of communications theory. In developing this approach, not only has full use been made of the extensive data acquisition and computing facilities available in this laboratory and in Colorado State University, but the expertise of various specialists has been called upon and freely given. The help of these individuals is gratefully acknowledged.

B. THE LONG-TERM EXPERIMENT: CURRENT STATUS AND FEASIBILITY STUDIES

The raison d'etre of the Collaborative Radiological Health Laboratory is the experiment designed to examine the long-term effects in the beagle of a single whole-body exposure at different periods of development. Six such periods have been chosen for study: 8, 28, and 55 days post-coitus and 2 days, 10 weeks, and 1 year. Exposure levels are 0, 20, or 100 R over a constant exposure time of 10 min. A total of approximately 1,700 animals will be involved. The first pups were entered into the experiment in December 1967 and the total assigned as of November 1969 was 456, rather more than would be predicted from the assumptions made when establishing commitment protocols. Assignment of animals will continue over the next 3 to 4 years.

Protocols have been developed for measuring a number of parameters in all or some of the long-term animals. For example, schedules have been developed for collecting data on body weight, head dimensions, and the time course of dental eruption in a sample population, and for assessing fertility in all males and breeding capacity in all females assigned to the experiment.

Although some assessment of the early effects of exposure of the pregnant bitch to 20 or 100 R will be possible in the near future, it will be many years before the data accruing from the long-term experiment can be fully evaluated. As is demonstrated by the preliminary results given on clinical examination of the eyes of over 100 of these dogs, the data collected to date are, in general, too scattered to warrant any attempt at analysis. These preliminary results do, however, tend to support the conclusion drawn from earlier experiments that eye lesions can be expected to be seen only in animals irradiated in utero. The relative insensitivity of the mature eye is evident from the paucity of significant lesions observed in animals receiving 100 to 1,000 R gamma radiation to one side of the head.

The long-term experiment is expected to last upwards of 20 years. In order to obtain a preview of events to be expected in animals reaching the end of their life-span, a pilot age-sensitivity study was initiated during 1968 for the purpose of following events for up to 4 years in animals which survived an exposure in the LD_{50} range at 8, 28, or 55 days post-coitus or 2 days post-partum. To November 1969, 117 of the projected total of 300 animals had been assigned to this experiment and 32 (scheduled for sacrifice at 70 days) had been necropsied.

Acute lethality studies have now progressed to the point where good estimates are available of the $LD_{50/60}$ at times between 2 days post-partum and 1 year; the conclusion can be drawn that the young beagle exhibits changes in radiosensitivity similar to those described in the mouse. Deaths at all postnatal ages-at-irradiation could be attributed primarily to the hematological syndrome.

A constant exposure time of 10 min., rather than a constant exposure rate, has been adopted for all exposures of long-term dogs and in many other studies. This has led to use of exposure rates of from 2 to 32 R per min. A small exposure rate study has shown that, although, as expected, LD_{50} values decreased as exposure rate was increased from 4 to 32 R per min.; this decrease is probably of minor concern in the context of the major studies being conducted at CRHL.

As the long-term experiment proceeds, methods will continue to be developed and refined to make the greatest possible use of the material and data available. A fruitful line of approach — and, indeed, an essential one — is the development of atraumatic methods for studying function. Preliminary investigations into auditory acuity in the beagle have now been undertaken. Very promising results have been obtained by analyzing acoustically evoked potentials recorded from the scalp using an on-line averaging computer. To allow comparison with more traditional methods, a behavioral avoidance acoustical conditioning technique is also being developed; the time-consuming nature of such methods is made plain by the preliminary studies.

An organ to which it is proposed to devote considerable effort in the long-term studies is the kidney. Preliminary work on optimal fixation techniques has been completed; fine-structure studies on kidneys from irradiated dogs are now underway. Further investigations have also been made into problems encountered with the single injection technique developed at CRHL for estimation of parameters of renal function. A contribution to external precordial counts from ^{125}I-hippuran accumulating in bile was found to be a significant factor, but this can be minimized by suitable positioning and collimation of the probe. Analysis of simultaneously-obtained blood and urine specimens could also offer a means of assessing certain aspects of renal function were it always possible to obtain urine from the dog. A simple electrical stimulation method has been developed for this purpose.

C. SHORT-TERM EFFECTS OF PRENATAL AND NEONATAL IRRADIATION

The phase of work in this laboratory which has taken second place only to the long-term experiment is definition of the acute injury following irradiation during early development.

The prenatal response of the dog to irradiation during the preimplantation, embryonic, and early fetal periods has been characterized. Exposure to 150 R ^{60}Co gamma radiation during the second half of the lengthy preimplantation period had no significant effect on prenatal mortality or on the incidence of developmental malformations. In contrast, a marked increase in prenatal mortality occurred when the same exposure was received shortly after implantation during the primitive streak stage and early organogenesis. Towards the end of the embryonic period the embryo was still quite sensitive in terms of lethal effects. The most sensitive time for production of malformations occurred during organogenesis but at a slightly later time than that for peak mortality.

Satisfactory progress has been made in introducing dogs into a study to assess recovery in the embryo, fetus, or neonate following exposure at 8, 25, or 55 days post-coitus or 2 days post-partum to 100 R ^{60}Co gamma radiation using a split-dose technique. This is one of a number of studies designed to absorb what would otherwise be "waste" dogs.

Fourier integral techniques have been applied to the analysis of electroencephalograms recorded from the scalp of young adult beagles which had been exposed to 390-500 R ^{60}Co gamma radiation as late fetuses. Differences from normal in both the power-spectral density and autocorrelogram could be detected in this way. A digital method, offering considerable advantages, for processing "random" continuous data has been developed for these and similar analyses; its use has been validated. These techniques are being pursued as a means of monitoring brain function in the long-term animals.

The effect of prenatal exposures in the LD_{50} range upon various aspects of kidney function has been examined using conventional techniques. These studies have provided information on changes in glomerular filtration rate, effective renal plasma flow, tubular maximum for p-aminohippurate, and concentrating capacity occurring during the first year of life. There is some suggestion that prenatal exposure may depress both GFR and Tm_{PAH} after about the 3rd month. Irradiated animals showed a depressed concentrating capacity but only during the first 2 weeks of postnatal life.

The glycerol phosphate dehydrogenase activity of fetal beagle liver and brain has been examined following exposure to 150 R ^{60}Co gamma radiation at various times during early development. In liver, but not in brain, a significant difference in response was observed in animals irradiated at 18 and 21 gestational days. This period corresponds with appearance of the liver in the beagle embryo suggesting that a direct effect upon the liver is involved.

In a further attempt to obtain information on loci of early radiation effects, again using enzyme ontogenesis as a model, factors affecting the rate of appearance of hepatic serine dehydrase in prematurely delivered rats have been investigated. These factors are now believed to be sufficiently understood to allow use of the system for assessing the effects of in utero irradiation. Rats are being used since they offer considerable economical advantages over dogs.

D. DEVELOPMENT OF THE BEAGLE DOG

From the numerous ancillary studies necessitated by the long-term experiment, data are accumulating on various aspects of normal development in the beagle. Of primary interest is characterization of the prenatal developmental stages derived from material which has been in process of collection since 1967. Timing of in utero development presents a fundamental problem in the dog and, contrary to initial expectations, use of the first day of metestrus as a reference point has been found to offer no easy solution.

Investigation of the practicability of measuring spinal growth in the living dog using radiographic techniques has given information on the growth of the first lumbar vertebra and the lumbar spine from 1 to 21 weeks post-partum.

Development of frequency components in the electroencephalogram over the first 6 months of life has been studied using power-spectral density measurements and applying a digital computation technique which permits analysis of ongoing activity. The adult frequency pattern is attained by about 48 days. Changes in the visual evoked potential over the same period have also been characterized and a transition period between the 14th and 30th day identified. In both instances, scalp recording was employed.

An elegant, on-line computer method, again using a noninvasive technique, has been evolved for monitoring pulse-wave velocity in the descending aorta of aging dogs. No difference in pulse-wave velocity was found in animals aged 6 months and 4-6 years.

Development of the glomerulus, proximal convoluted tubule, and adjacent interstitial tissue in the kidney of the young dog has been examined using light and electron microscope techniques. Mesangial tissue is present and readily identifiable by the 2nd postnatal day; nephrogenic tissue persists until at least the 12th day. From the first week to the 6th month of life, reabsorption of inorganic phosphate by the renal tubules has been found to be high and to approach the tubular maximum.

E. LABORATORY MANAGEMENT

The cesarian-derived, controlled-access beagle colony provides the experimental material for this project. During 1968 the colony increased in size to approximately 1,900 animals of which some 1,350 were assigned to experimental studies; the remainder were mainly breeding stock or weanling pups. There has been little change either in management of the colony, apart from slight modification of the vaccination schedule to avoid concomitant medical and experimental insult, or in medical and surgical conditions encountered. The introduction, in 1968, of a program for selecting breeding males has resulted in a satisfactory increase in conception rate (to 87 percent in unirradiated females).

With the exception of a small number of abortions (none associated with *Brucella canis*) there has also been no significant change in the pattern of neonatal mortality. Two cases of polyarthritis, a new disease for the colony, were, however, encountered in older dogs. Clinical and pathological findings in two bitches dying near the time of whelping were suggestive of pregnancy toxemia, hitherto not reported in the bitch. Investigation of the possible association of ^{125}I-hippuran (used in kidney function studies) with renal inflammation revealed an unsuspected increase in the incidence of this condition in young beagles over the past 3 years. The cause is not known. Thyroiditis, cardiopulmonary disease, and glomerulonephritis remain the important conditions occurring spontaneously in the colony.

Continuing the series of reports on spontaneous conditions in young puppies, hepatic abscessation, sporadically encountered in the CRHL colony, has been characterized. Probably of omphalogenic origin, the causal organism identified is usually staphylococcus or, less frequently, streptococcus. However, gram-positive, filamentous organisms believed to be actinomycetes were found in 4 of the 22 cases reported.

The ancestral composition of the colony has now been analyzed in terms of a base colony defined as those beagles originally purchased whose descendants were present in the colony as of November 1969. The policy has been to out-breed rather than to attempt in-breeding and a satisfactory degree of heterogeneity appears to have been achieved.

During the year, the operations of the Biometry Section, which plays a key role in laboratory management, have been thoroughly reviewed. An inventory has been prepared of data listings found to be necessary for satisfactory communications and house-keeping within CRHL.

A defect (since rectified) associated with the automatic switching circuit in the ammeter of a TLD reader to be used in routine dosimetry was encountered during calibration of the instrument. The defect could have led to a possible error of ± 10 percent in estimation of exposures in the order of 100 R.

APPENDIX A

PUBLICATIONS JANUARY - DECEMBER 1969

Abuelo, J.G. and D.E. Moore. 1969. The human chromosome: Electron microscopic observations on chromatin fiber organization. J. Cell Biol. 41:73-90.

Anderson, G.E., R.A. Tell, and H.D. Youmans. 1969. Spectral transmission and attenuation of X-radiation by glasses and resins used in color television picture tubes, p. 743-748. In Proceedings of the National Electronics Conference, Volume XXV, National Electronics Conference, Inc., Oak Brook, Illinois 60521.

Angleton, G.M., B.H. Peterson, A.C. Lee, and R.J. Garner. 1969. LD-50 and dose rate studies in beagles. Radiation Res. 39(2): 473.

Barth, J. and B.H. Bruckner. 1969. Evaluation of clays as binding agents for reduction of radionuclides in milk. Binding properties of natural and hydrogen form clays with strontium and essential cations in artificial rumen and in simulated abomasal and intestinal fluids. J. Agr. Food Chem. 17(6):1340-1343.

Barth, J. and B.H. Bruckner. 1969. Evaluation of clays as binding agents for reduction of radionuclides in milk. Binding properties of clays with ^{134}Cs in artificial rumen and in simulated abomasal and intestinal fluids, and uptake of ^{134}Cs by rumen microflora. J. Agr. Food Chem. 17(6):1344-1346.

Barth, J., A.N. Mikalis, J.Y. Harris, and B.H. Bruckner. 1969. Evaluation of clays as binding agents for reduction of radionuclides in milk. Effect of Belle Fourche bentonite on excretion of ^{134}Cs in lactating goats. J. Agr. Food Chem. 17(6):1347-1349.

Berman, E. and L. Rosenstein. Sound and infrasound (Division of Biological Effects, Bureau of Radiological Health, Rockville, Maryland 20852), March 1969, 18 p. (for internal use only).

Bureau of Radiological Health, Population Exposure Studies Section. Population dose from X-rays, U.S. 1964. Estimates of gonad and genetically significant dose from the Public Health Service X-ray exposure study. PHS Publication No. 2001, October 1969, 143 p.

Cisar, C.F. 1969. The rat kangaroo (Potorous tridactylus). Handling and husbandry practices in a research facility. Lab. Animal Care 19(1): 55-59.

PRECEDING PAGE BLANK

Davis, A.K., S.J. Smith, and J.W. Laskey. 1969. Kidney function in aging irradiated monkeys. Radiation Res. 39(2): 467.

Ellingson, D.J. and K.T.S. Yao. 1969. Chinese hamster testicular cells cultivated in vitro, p. 149. *In* Hemic Cells in Vitro, A Symposium of the Tissue Culture Association, Vol. 4, P. Farnes (ed.), Williams and Wilkins Co., Baltimore.

Hazzard, D.G. 1969. Percent cesium-134 and strontium-85 in milk, urine, and feces of goats on normal and verxite-containing diets. J. Dairy Science 52(7):990-994.

Hazzard, D.G., T.J. Withrow, and B.H. Bruckner. 1969. Verxite flakes for in vivo binding of cesium-134 in cows. J. Dairy Sci. 52(7):995-997.

Kahn, B., C.P. Straub, P.J. Robbins, H.N. Wellman, R.A. Seltzer, and N.C. Telles. 1969. Retention of radiostrontium, calcium, and phosphorus by infants. Pediatrics 43, Suppl. 4, Part II:651-756.

Key, C.R., R.J. Sampson, C.R. Buncher, H. Oka, and S. Iijima. 1969. Association of thyroid carcinomas with other primary cancers, Hiroshima-Nagasaki. Atomic Bomb Casualty Commission Technical Report Series TR 18-69.

Levin, S.G. and R.K. Stoms. 1969. Natural background gamma radiation dose rate measurements in Michigan, Colorado, and Minnesota. Am. J. Public Health 59(1):102-109.

Maletskos, C.J., A.T. Keane, N.C. Telles, and R.D. Evans. 1969. Retention and absorption of ^{224}Ra and ^{234}Th and some dosimetric considerations of ^{224}Ra in human beings, p. 29-49. *In* Delayed Effects of Bone-Seeking Radionuclides, C.W. Mays, et al. (eds.), Univ. of Utah Press, Salt Lake City.

Moore, W., Jr. 1969. Biological aspects of laser radiation - A review of hazards (Bureau of Radiological Health, Environmental Control Administration, Rockville, Maryland 20852,)January 1969, 15 p.

Nelson, N.S., E. Berman, and J.F. Stara. 1969. Litter size and sex distribution in an outdoor feline colony. Carnivore Genet. Newsletter No. 8:181-191.

Nelson, N.S. and R.M. Hoar. 1969. A small animal balling gun for oral administration of experimental compounds. Lab. Animal Care 19(6):871-872

Phemister, R.D., J.N. Shively, and S. Young. 1969. The effects of gamma irradiation on the postnatally developing canine cerebellar cortex. I. Effects of single sublethal exposures. J. Neuropathol. Exp. Neurol. XXVIII(1):119-127.

Phemister, R.D., J.N. Shively, and S. Young. 1969. The effects of gamma irradiation on the postnatally developing canine cerebellar cortex. II. Sequential histogenesis of radiation-induced changes. J. Neuropathol. Exp. Neurol. XXVIII(1):128-138.

Phemister, R.D. and S. Young. 1969. The postnatal development of the canine cerebellar cortex. J. Comp. Neurol. 134(2):243-254.

Sampson, R.J., C.R. Key, C.R. Buncher, and S. Iijima. 1969. Thyroid carcinoma in Hiroshima and Nagasaki. 1. Prevalence of thyroid carcinoma at autopsy. J. Am. Med. Assoc. 209:65-70.

Sampson, R.J., C.R. Key, C.R. Buncher, and S. Iijima. 1969. The age factor in radiation carcinogenesis of the human thyroid. Atomic Bomb Casualty Commission Technical Report Series TR 7-69.

Sampson, R.J., C.R. Key, C.R. Buncher, H. Oka, S. Iijima. 1969. Papillary carcinoma of the thyroid: Sex and size related features. Atomic Bomb Casualty Commission Technical Report Series TR 8-69.

Sampson, R.J., H. Oka, C.R. Key, C.R. Buncher, and S. Iijima. 1969. Metastases from occult thyroid carcinoma - An autopsy study from Hiroshima and Nagasaki, Japan. Atomic Bomb Casualty Commission Technical Report Series TR 16-69.

Shively, J.N. and G.P. Epling. 1969. Fine structure of the thyroid gland of young dogs. Am. J. Vet. Res. 30:219-228.

Shively, J.N. and G.P. Epling. 1969. Fine structure of the canine eye: Iris. Am. J. Vet. Res. 30(1):13-25.

Shively, J.N. and G.P. Epling. 1969. Early changes in the fine structure of the thyroid of midlethally irradiated dogs. Radiation Res. 37:71-82.

Shively, J.N., C. Feldt, and D. Davis. 1969. Fine structure of formed elements in canine blood. Am. J. Vet. Res. 30(6):893-905.

Shively, J.N., R.D. Phemister, and G.P. Epling. 1969. The fine structure of the thyroid epithelium of young dogs treated with thyrotropin. Am. J. Vet. Res. 30(2):229-241.

Smith, R.S., H. Hoffman, and C.F. Cisar. 1969. Congenital cataract in the rat. Arch. Ophthalmol. 81:259-263.

Stara, J.F. and D. Waldron-Edward. 1969. Repressive action of sodium alginate on absorption of radioactive strontium and calcium in cats, p. 340-354. In Diagnosis and Treatment of Deposited Radionuclides, Proceedings of a Symposium held at Richland, Washington, 15-17 May, 1967. Excerpta Medica Foundation.

Telles, N.C. and B.C. Ward. 1969. The effects of radiation and ethionine on rat mammary tumor incidence. Radiation Res. 37(3):577-589.

Telles, N.C., B.C. Ward, E.A. Willensky, and G.L. Jessup. 1969. Radiation-ethionine carcinogenesis, p. 233-245. In Radiation-Induced Cancer, Proceedings of a Symposium, Athens, 28 April - 2 May 1969, International Atomic Energy Association, Vienna.

Tompkins, E. and M.L. Brown. 1969. Evaluation of a possible causal relationship between fallout deposition of strontium-90 and infant and fetal mortality trends (DBE 69-2) (Division of Biological Effects, Bureau of Radiological Health, Rockville, Maryland 20852), October 1969. 37 p.

Ward, B. C. and W. Moore, Jr. 1969. Spontaneous lesions in a colony of Chinese hamsters. Lab. Animal Care 19(4):516-521.

Wolfangel, R.G. and J.F. Stara. 1969. Sequential determinations of the in situ distribution of ^{131}I-labeled tetracycline in cats using the scintillation camera. J. Nuclear Med. 10(11):697-701.

Yao, K.T.S. 1969. Radioprotective effect of rat kangaroo haematopoietic cells on X-irradiated mice and Chinese hamsters. Intern. J. Radiation Biol. 15(2):191-194.

Yao, K.T.S. and D.J. Ellingson. 1969. Observations on nuclear rotation and oscillation in Chinese hamster germinal cells in vitro. Exp. Cell Res. 55:39-41

APPENDIX B

PAPERS PREPARED FOR PRESENTATION
JANUARY - DECEMBER 1969

Anderson, G.E., R.A. Tell, and H.D. Youmans. Spectral transmission and attenuation of X radiation by glasses and resins used in color television picture tubes. Health Physics Society, Pittsburgh, Pennsylvania, June 8-12, 1969. Also presented at National Electronics Conference, Chicago, Illinois, December 8-10, 1969.

Angleton, G.M. Alternate methods for obtaining estimates of lethality probabilities in LD-50 studies. Health Physics Society, Pittsburgh, Pennsylvania, June 8-12, 1969.

Angleton, G.M. A generalized mathematical model for biological recovery with applications in radiation biology. Joint Statistical Meetings of the American Statistical Association, Biometric Society, and Institute of Mathematical Statistics, New York, August 19-22, 1969.

Angleton, G.M., B.H. Peterson, A.C. Lee, and R.J. Garner. LD-50 and dose rate studies in beagles. Radiation Research Society, Cincinnati, Ohio, May 18-22, 1969.

Angleton, G.M., C.D. Robinette, and J. de Boer. Quantitative aspects of recovery. Second International Conference on Medical Physics, Boston, Massachusetts, August 11-15, 1969.

Awa, A., M. Yoshida, T. Sofuni, A. Bloom, Y. Ii, K. Iishida, T. Yamamoto, and D.H. McGregor. Cytogenetic studies of tissues obtained at autopsy. 14th Meeting of Japanese Society of Human Genetics, Sapporo, Japan, September 1-3, 1969.

Beyer, E.C. Biological effects of microwaves and lasers. Regional Meeting of Health Physicists, Evanston, Illinois, May 3, 1969.

Bloom, A.D., A.A. Awa, and S. Neriishi. A-bomb exposure and chromosome aberrations: Implications of the findings from Hiroshima and Nagasaki. Radiation Research Society, Cincinnati, Ohio, May 18-22, 1969.

Childress, J.R., B.C. Ward, and J.N. Shively. Spontaneous lymphosarcoma in a colony of Long-Evans rats. American Association of Laboratory Animal Science, Dallas, Texas, October 13-17, 1969.

Cisar, C.F. Management of a breeding colony of Chinese hamsters. Upstate New York Branch, American Association for Laboratory Animal Science, Rochester, New York, May 24, 1969.

Cisar, C.F., E.P. Gumperz, F.S. Nicholson, and W. Moore, Jr. A practical method for production breeding of Chinese hamsters. American Association for Laboratory Animal Science, Dallas, Texas, October 13-17, 1969.

Davis, A.K., S.J. Smith, and J.W. Laskey. Kidney function in aging irradiated monkeys. Radiation Research Society, Cincinnati, Ohio, May 18-22, 1969.

Davis, A.K., J.D. Verley, and K.N. Juneau. Altered development of kidney function following _in utero_ irradiation. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Dobyns, B.M., G.E. Sheline, J.B. Workman, E.A. Tompkins, W.M. McConahey, and D.V. Becker. Incidence of benign and malignant lesions of the thyroid in patients treated for hyperthyroidism. (A report of the Cooperative Thyrotoxicosis Therapy Follow-Up Study). American Thyroid Association, Chicago, Illinois, November 12-15, 1969.

Garner, R.J., J.P. Graves, and C.E. Lane. Irradiation and enzyme ontogenesis in the rat and beagle. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Gillespie, L.J., W.M. Leach, and B.C. Ward. In vitro culture of Chinese hamster leukocytes. Fourth Leukocyte Culture Conference, Dartmouth Medical School, Hanover, New Hampshire, June 26-28, 1969.

Harris, J.Y. and M.M. Jiles. Radiation and solid wastes as environmental hazards. Washington Saturday College, Washington, D.C., March 15, 22, April 12, 19, 1969.

Hazzard, D.G., and R.A. Budd. Effects of _in utero_ radiation on the peripheral blood of the neonatal rat. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Hemphill, F.M., D. Erickson, A. Hoffman, K. Yednock, and R. Connors. Trends in general hospital utilization of radiation. Fourth Annual Joint Meeting of the Commissioned Officers Association and Clinical Society, Boston, Massachusetts, May 8-10, 1969.

Hemphill, F.M., and R.D. Hesselgren. Thorotrast quantities and whole-body counting. Fourth Annual Joint Meeting of the Commissioned Officers Association and Clinical Society, Boston, Massachusetts, May 8-10, 1969.

Janes, D.E., W.M. Leach, W.A. Mills, R.T. Moore, and M.L. Shore. Research interests in the biological effects of microwave radiation. Radio and Microwave Radiations Applications and Potential Hazards Symposium, Guildford, England, January 2-3, 1969.

Kamat, G.P. and D.E. Janes. Studies on the effect of 2450 MHz microwaves on human immunoglobulin G. Symposium on the Biological Effects and Health Implications of Microwave Radiation, Richmond, Virginia, September 17-19, 1969.

Lambert, P.D., R.J. Morgan, G.M. Angleton, and W.J. Tietz. Determination of propagation direction in peripheral nerve. Sixth Annual Rocky Mountain Bioengineering Symposium, Laramie, Wyoming, May 5-6, 1969.

Lambert, P.D. and W.J. Tietz. Development of the average visual evoked potential in the beagle. Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 13-16, 1969.

Leach, W.M. Electron microscopy of chromosomes. Lecture, Department of Zoology, George Washington University, Washington, D.C., May 7, 1969.

Lee, W. Doses in the brain, eye, and thyroid of children from 60-110 kVp X-ray epilation for tinea capitis. Second International Conference on Medical Physics, Boston, Massachusetts, August 11-15, 1969.

Lee, W. Beam irradiation of the rat thyroid. Second International Conference on Medical Physics, Boston, Massachusetts, August 11-15, 1969.

Mack, J.F., H.N. Wellman, R.L. Witt, and R.H. Poe. Pulmonary perfusion - Ventilation lung studies. Society of Nuclear Medicine, New Orleans, Louisiana, June 25-28, 1969.

Manes, J.D., R.P. Chiacchierini, and H.D. Youmans. A chromatographic analysis of the lipids of beagle pup brain exposed in vivo to X radiation. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Mills, W.A. Biological effects of non-ionizing radiation from electronic products. Conference on Implementation of Public Law 90-602, Montgomery, Alabama, March 23-26, 1969.

Modrick, J.A. Visual discrimination learning in normal and neonatally irradiated beagles. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Morgan, R.J., C.C. Turbes, and P.D. Lambert. Autocorrelograms of CNS disorders in the canine. Sixth Annual Rocky Mountain Bioengineering Symposium, Laramie, Wyoming, May 5-6, 1969.

Nankin, H.R. and J.G. Abuelo. The centromere of human lymphocyte chromosomes during colcemid mitosis. American Federation of Clinical Research, Atlantic City, New Jersey, May 4-5, 1969.

Nelson, N.S., J.F. Stara, and R. Hoar. Concentration and dose distribution of radioiodine in embryonic guinea pigs during early embryogenesis. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Penfil, R.L. and M.L. Brown. Genetically significant dose to the U.S. population from diagnostic medical roentgenology. XII International Congress of Radiology, Tokyo, Japan, October 6, 1969.

Phemister, R.D., R.J. Garner, J.N. Shively, and W.J. Tietz. The radiosensitivity of the developing beagle. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Sampson, R.J., C.R. Key, C.R. Buncher, and S. Iijima. Thyroid carcinoma in Hiroshima and Nagasaki. Symposium on Thyroid Disease, Sections of Pathology and Internal Medicine, New York, N.Y., July 14, 1969.

Sampson, R.J., C.R. Key, C.R. Buncher, and S. Iijima. The age factor in radiation carcinogenesis of the human thyroid. Ninth Annual Hanford Symposium, Richland, Washington, May 5-8, 1969.

Sampson, R.J., C.R. Key, C.R. Buncher, H. Oka, and S. Iijima. Cervical lymph node metastases from occult carcinoma of the thyroid. American Society of Clinical Pathologists, Chicago, Illinois, September 14-20, 1969.

Schreiber, W.M., H. Kato, and J.D. Robertson. Carcinoma of the liver and cirrhosis of the liver: Their relationships to each other and to ionizing radiation. Japan Gastroenterological Society, Sendai, Japan, April 4-6, 1969.

Sheline, G.E., B.M. Dobyns, J.B. Workman, E.A. Tompkins, W.M. McConahey, and D.V. Becker. Incidence of malignant lesions of the thyroid in patients treated for hyperthyroidism. Nuclear Medicine Section, American Medical Association, New York, July 14, 1969.

Shively, J.N. Development of a beagle colony at the Collaborative Radiological Health Laboratory at Fort Collins. Fort Detrick Veterinary Luncheon, Fort Detrick, Maryland, January 17, 1969.

Shively, J.N., R.D. Phemister, and G.P. Epling. Radiation-induced alterations in the ultrastructure of the developing and mature mammalian eye. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Shore, M.L. J.W. Laskey, and L.R. Simmons. Impaired protein synthesis in the fetally irradiated rat. Ninth Annual Hanford Biology Symposium, Richland, Washington, May 5-8, 1969.

Sodd, V.J. and K.L. Scholtz. ^{123}I production with the compact cyclotron. Society of Nuclear Medicine, New Orleans, Louisiana, June 25-28, 1969.

Stara, J.F., E. Berman, and M. Replogle. Laboratory cat induction process in an outside colony. American Association for Laboratory Animal Science, Dallas, Texas, October 13-17, 1969.

Telles, N.C., B.C. Ward, E.A. Willensky, and G.L. Jessup. Radiation-ethionine carcinogenesis. Symposium on Radiation-Induced Carcinogenesis, Athens, Greece, April 28 - May 2, 1969.

Thoma, G.E., E.A. Tompkins, and E.L. Saenger. Incidence of lymphoma and leukemia after therapy of hyperthyroidism (follow-up report). Nuclear Medicine Section, American Medical Association, New York, July 14, 1969.

Tompkins, E. Late effects of radioiodine therapy. Oak Ridge Associated Universities Symposium in Medicine, No. 12: Medical Radionuclides - Radiation Dose and Effects, Oak Ridge, Tennessee, December 9-11, 1969.

Wellman, H.N., J.F. Mack, E.L. Saenger, and V.J. Sodd. A study of the parameters influencing the clinical use of ^{123}I. Society of Nuclear Medicine, New Orleans, Louisiana, June 25-28, 1969.

Yamamoto, T., K. Ishida, H. Kato, D.H. McGregor, and E. Tahara. Gastric carcinoma in A-bomb survivors, Hiroshima and Nagasaki. 28th Annual Meeting of the Japanese Cancer Association, Kanazawa, Japan, October, 1969.

Yamamoto, T., K. Ishida, D.H. McGregor, E. Tahara, and H. Kato. Study of gastric carcinoma in a fixed population, Hiroshima and Nagasaki. 58th Annual Meeting of the Japanese Pathological Society, Fukuoka, Japan, April 1-3, 1969.

Yao, K.T.S. and M.M. Jiles. Effects of microwave radiation on cultivated rat kangaroo cells. Symposium on the Biological Effects and Health Implications of Microwave Radiation, Richmond, Virginia, September 17-19, 1969.

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January - December 1969 (DBE 70-1)
Division of Biological Effects, Bureau of Radiological Health, DHEW.

This report summarizes the activities of the Division of Biological Effects during 1969. The investigations of Epidemiologic Studies Branch and the Experimental Studies Branch which comprise the Division are discussed. Titles of papers that Division investigators have prepared for presentation and those appearing in print during the year are listed.

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X-ray, Radiobiology, Microwave, Radiation research, Radionuclides, Human radiation effects, Long-term effects, Nonionizing radiation

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